THE UNIVERSITY OF LIVERPOOL

ANNALS

OF

TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by

Professor Warrington Yorke, M.D., F.R.C.P., F.R.S.

Professor D. B. Blacklock, M.D., D.P.H.

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Professor T. H. Davey, M.D., D.T.M.

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(March 31st, 1939, to December 30th, 1939)

With frontispiece, five plates, two maps, four charts, and sixty eight figures in text

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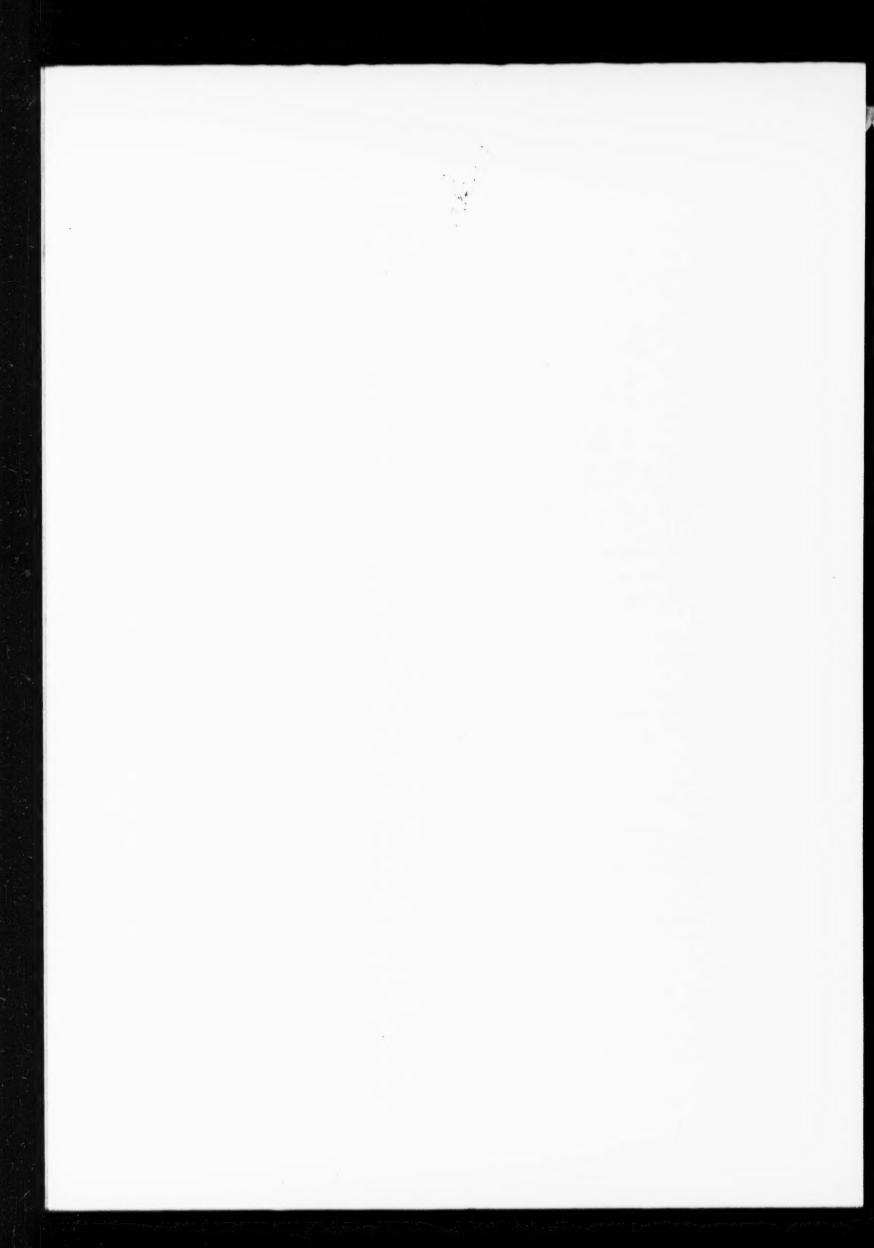
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March 31st, 1939

Vol. 33. No. 1



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1912	Anderson, Edmund Litchfield	1914	Naidu, Bangalore Pasupulati Balakrishna
1912	Borle, James	1914	Rowe, John Joseph Stephen
1912	Bowie, John Tait	1914	Roy, Raghu Nath
	Brassey, Laurence Percival Christie, David	1914	Shiveshwarkar, Ramchandra Vishnu
	Dillon, Henry de Courcy		Sur, Sachindra Nath
	Dunn, Lillie Eleanor		Talati, Dadabhai Cursedji Wilkingan, Arthur Geden
	Hardwicke, Charles		Wilkinson, Arthur Geden Wright, Ernest Jenner
1912	Jagose, Jamshed Rustomji	1019	wingin, Linest Jenner
1912	Kochhar, Mela Ram		Lobo, John Francis
	McGusty, Victor William Tighe		Madhok, Gopal Dass
	Milne, Arthur James		Pearson, George Howorth
	Mitra, Manmatha Nath		Swami, Karumuri Virabhadra
1912	Myles, Charles Duncan	1915	Wood, John

Date of Diploma		Date of Diploma	
1916	Barseghian, Mesroob	1922	
1916	Chaliha, Lakshmi Prasad	1922	Rieley, Stanley Desmond
1916	Lim, Albert Liat Juay	1922	Rutherford, Gladys Stewart, Quintin
1916	Lim, Harold Liat Hin		
1916	Metzger, George Nathanie	$\frac{1923}{1923}$	Abelman, B.
1916	Söderström, Erik Daniel	1923	Basu, Dhirendranath Cruickshank, John Cecil
1916	Wheeler, Louis	1923	Doherty, Winifred Irene
1917	Chapman, Herbert Owen	1923	Edghill, Winifred M.
1917	Krishnamoorthy, Yedatore Venkoba	1923	Elsohn, John
1917	Lipkin, Isaac Jacob	1923	Fraser, N. D.
1918	Watts, Rattan Claud	1923	Lee, R.
1919	Bowle-Evans, Charles Harford	1923	Peirce, E. R.
1919	Burnie, Robert McColl	$\frac{1923}{1923}$	Raja, Rojaporum
1919	Celestin, Louis Abel	1923	Reid, C. B. B. Richmond, A. E.
1919	Cummings, Eustace Henry Taylor	1923	Steven, J. B.
1919	Darling, Georgina Renington	1923	White, Charles Francis
1919	Drake, Joan Margaret Fraser	1924	Bilimoria, H. S.
1919 1 91 9	Fraser, William James	1924	Carson, J. C.
1919	Gordon, Rupert Montgomery Krige, Christian Frederik	1924	Chopra, B. L.
1919	Maplestone, Philip Alan	1924	Davis, B. L.
1919	Oluwole, Isaac Ladipo	1924	Hardy, M. J.
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1919	Sawers, William Campbell	1924	Johnstone, F. J. C.
1919	Thompson, Mary Georgina	$\frac{1924}{1924}$	Keirans, J. J.
1919	Turner, Gladys Maude	1924	Lee, S. W. T Macdonald, G
1919	Young, Charles James	1924	Maclean, G.
1920	Adler, Saul	1924	Mathur, W. C.
1920	Anderson, William Jenkins Webb	1924	Mitchell, J. M.
$\begin{array}{c} 1920 \\ 1920 \end{array}$	Campbell, George	1924	Owen, D. Uvedale
1920	Cobb, Charles Eric Cobb, Enid Margaret Mary	1924	Palmer-Jones, Beryl
1920	Connolly, Evelyn Mary	1924	Sankeralli, E. J.
1920	Fernandez, Daniel David	$\frac{1924}{1924}$	Singh, H.
1920	Lim, Chong Eang		Theron, Elizabeth M.
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1920	van der Merwe, Frederick	$\begin{array}{c} 1925 \\ 1925 \end{array}$	Ashton, Frank Richard Ashworth, Esther
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1921	Longhurst, Bell Wilmott	$\begin{array}{c} 1925 \\ 1925 \end{array}$	Crawford, E. J.
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1921	Madan, Hans Raj	1925	Fisher, Morris
1921	Mulligan, William Percival	1925	Green, Frederick Norman
$\begin{array}{c} 1921 \\ 1921 \end{array}$	Nixon, Robert	1925	Grutu, M. S.
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1921	Thomson, Marion	$\begin{array}{c} 1925 \\ 1925 \end{array}$	Kerr, James R.
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1922	Lethem, William Ashley	1925	Skan, Douglas A.
$\begin{array}{c} 1922 \\ 1922 \end{array}$	Paul, Sachchidananda Hoshen	$\begin{array}{c} 1925 \\ 1925 \end{array}$	Stone, Ernest R.
1022	Pinder, John	1920	Terrel, C. G.

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1925 1925	Thompson, C. H. B. Tooth, Frederick	1927 1927	Bawa, H. S. Bilimoria, J. D.
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1927	Bahl, M. L.	1928	Blakemore, W. L.
1927	Barrowman, B.	1928	Camps-Campins, J. M.

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1930	Wilson, T.	1933	Ghosh, M. M.
	***************************************	1933	Greson, L. P.
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1931	Kuo, J. T.	1933	Zan, M. S.
1931	Kuruvila, P. K.	1094	A 1 1 A
1931	Lakhwarah, M.	1934	Ahmed, A.
1931	Low, E. W.	1934	Amzalak, E. S.
1931	Maass, E. W. H.	1934 .	Appleton, S. K.
1931	McNair, H.	1934	Arulpragasam, A. R.
1931	Maniar, H. A.	1934	Awoliyi, S. O.
1931	Mansur, J.	1 934 1934	Beausang, J. J.
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1931	Moir, K. T.	1934	Chang, P. Y.
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1931	Sardana, M. N.	1934	Dunlop, R. Y.
1931	Sharma, D. R. Speirs, R. C.	1934	Edwards, A. C.
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1931	Yunibandhu, J.	1934	Hughes, W.
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1932	Adams, H. E. M.	1934	Kallianpurkar, S. L.
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1932	Bhandari, B. N.	1934	McElney, J. H.
1932	Bindra, B. S.	1934	McMillan, J. S.
1932	Bowesman, R.	1934	Macpherson, D. C. M.
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1932	Doherty, J. A.	1934	Purcell, P. J.
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1932	Ling, L. C.	1934	Tay, K. S.
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1932	Nirodi, B. S. R.	1934	Wu, T. T.
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1932	Zau, F. D.	1935	Harmer, Freda
		1935	Kamel, A.
1933	Amirtha Nayagam, G. P. A.	1935	Khalil, M. A. M.
1933	Anderson, N. E. W.	1935	Kirshner, A.
1933	Armah, J. E.	1935	Lahiri, M. N.
1933	Athavale, V. B.	1935	Li, C. H.
1933	Chan, L. F.	1935	Mahallawy, A. H. S.
1933	Day, F. M.	1935	Morton, Marjorie B.

Date of		Date of Diploma	1
•			
1935	Nasr, M. A.	1937	Hamid, M. S. B. A.
1935	O'Connor, R. J.	1937	Hutchinson, Roberta I.
1935	O'Toole, K.	1937	Khan, M. U.
1935	Patel, R. V.	1937	Kok, O. V. S.
1935	Robertson, J. R.	1937	Kotak, C. H.
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1935	Ting, S. K.	1937	Ludlam, G. B.
1935	el Tobgy, M. A.	1937	Mahadevan, M. N.
1935	Tomlinson, Mary A.	1937	Manwell, W.
1935	Tu, T. P.	1937	Mathew, K. C.
1935	Walker, A. J.	1937	Mazzotti, L.
1935	Weeks, E. B.	1937	Patwardhan, V. G.
		1937	Prasad, H.
1 (141) (4		1937	Robert, S. L.
1936	Amegatcher, J. E. O.	1937	Siddique, Y. M.
1936	Barnetson, W.	1937	Singh, N. N.
1936	Bhaduri, D. N.	1937	Syed, S. H. R.
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1936	Bradbury, E.	1937	West, H. F.
1936	Chakravarti, N.	1937	Wilson, J. D. T.
1936	Cran, D. L.	1937	Wislicki, Luise
1936	Easmon, R. S. A.	1937	Yofé, J.
1936	Gandhi, N. G.	1938	Affara, A. S.
1936	Ghoshal, R. G.	1938	-
1936	Gomaah, M. H.	1938	Bastawros, F. Basu, B.
1936	Green, B. J.	1938	
1936	Hazra, S.		Bourke, P. J.
1936	Hiremath, A. G.	1938 1938	Campbell, S. J.
1936	Ho, Chin Lien.	1938	Charles, L. J.
1936	Hurtado, L.		Chatterjee, S. K.
1936	Ismail, M. T.	1938	Cowper, S. G.
1936	Mukerjee, B. B.	1938	Einstein, O.
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1936	Tabet, A.	1938	Kay, J.
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1937	Daley, E. A.	1938	Shah, M. H.
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1937	Ghose, S. K.	1938	Watt, G. C.

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Date of		Date of	
Diploma		Diploma	
1926	Aitken, W. J.	1926	Lennox, D.
1926	Bligh-Peacock, N.	1926	Mackay, A. G.
1926	Clark, G.		Mackay, D. M.
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1926	Cullen, T.		MacSweeney, M.
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1926	Hawe, A. J.	1926	Turnbull, N. S.

Date of Diplom		Date of Diplom	
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1927	Besson, W. W.	1931	Verghese, G.
$\frac{1927}{1927}$	Dunlop, G. A.	1000	n C D E
	Earl, J. C. St. G.	1932	Bowers, G. P. F.
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1928	Morley, A. H.	1304	Silva, C. W. A. de
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1928	Pottinger, J. H.	1935	Awoliyi, S. O.
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1930	Baxter, G. R.	1000	14, 1. 1.
1930	Booker, C. G.	1937	Amagatcher I E ()
1930	Bullen, W. A.	1937	Amegatcher, J. E. O. Bradbury, E
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1930	Wilson, T.	1001	Wisher, Luise
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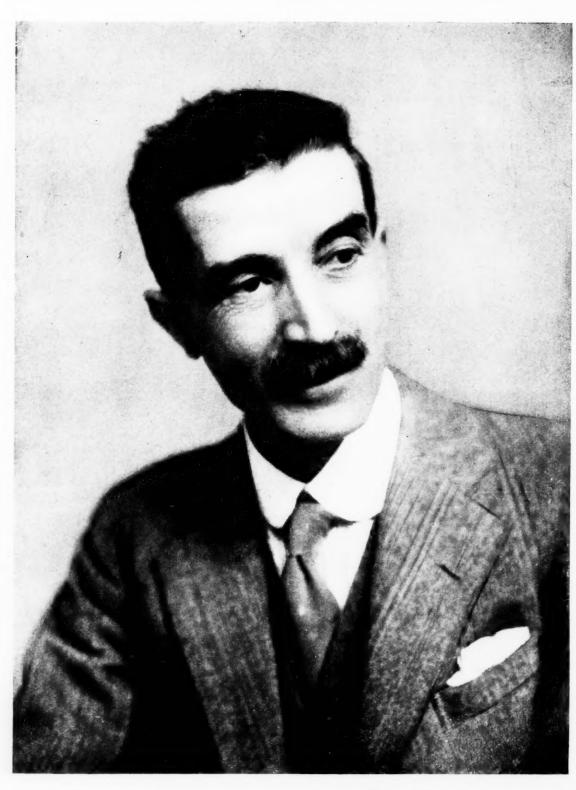
References to authors in the text must be made in the following way:—'According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.' The references should be collected in alphabetical order of author's surnames at the end of the paper, and arranged in the following way:—

ROBINSON, S. (1914). The spleen in malaria. Ann. Nosology, 20, 20.

SMITH, J. (1900). Enlargement of the spleen in malaria. Il. Pathometry, 1, 1.

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THE CHEMOTHERAPEUTIC REACTIONS OF RELAPSING FEVER SPIROCHAETES IN VITRO

BY

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(Received for publication July 26th, 1938)

The present paper describes an investigation of the chemotherapeutic reactions of relapsing fever spirochaetes by the *in vitro* methods which have been developed in recent years for the study of trypanosomes. Attention has been directed in particular to measurement of the spirochaeticidal action of drugs *in vitro*, and to the fixation of arsenical compounds by the spirochaetes.

The strain of spirochaetes used was one kindly supplied by Professor H. Schlossberger, of Berlin, who states that it is an old laboratory strain of unknown origin, which is more sensitive to arsenicals that the other strains in his possession, and which is apparently not capable of infecting man. It is the same strain as that used by Feldt and Singer during recent years. In mice it produces very heavy infections, which are seldom fatal, but it develops poorly in rats.

The full chemical names of the compounds used are as follows:

1. Reduced tryparsamide—disodium di(carboxy-methyl) 4-glycineamido-phenyl-thio-arsinite.

2. Phenyl-arsenoxide (dissolved in sodium hydroxide).

3. Reduced stovarsol—disodium di(carboxy-methyl) 4-hydroxy-3-acetamino-phenyl-thio-arsinite.

4. Reduced orsanine—disodium di(carboxy-methyl) 2-hydroxy-4-acet-

amino-phenyl-thio-arsinite.

5. Reduced neocryl—disodium di(carboxy-methyl) 4-succinanilo-methylamide-phenyl-thio-arsinite.

6. K.352—di - glutathionyl - 4 - acetamino - 2 - hydroxyphenyl - thio - arsinite (sodium salt).

7. Parosan oxide—8-acetamino-3-oxo-1, 4-benzisoxazin-6-arsenoxide.

8. P-carboxy-phenyl dichlorarsine (dissolved in sodium hydroxide).

9. Novarsenobillon—sodium 3:3' diamino-4:4' dihydroxy-arseno-benzene methylene sulphoxylate.

10. Arsenious oxide (dissolved in sodium hydroxide).

11. Tartar emetic—antimony potassium tartrate.

12. Diamino-methyl-acridine—2:8 diamino-10 methyl-acridinium-chloride hydrochloride (this is the main constituent of the pharmaceutical preparations of 'acriflavine', in which it is diluted by 15-40 per cent. of 2:8 diamino-acridine hydrochloride; see Hawking, 1938a).

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13. Solganal — disodium *p*-sulphomethyl-amino-*o*-auro-mercaptobenzene sulphonate.

Solganal-Resistant Strain

To facilitate the intended research, it was decided to prepare a solganal-fast strain of spirochaetes by exposing the parasites repeatedly to the compound in vivo. By this means, Fischl and Singer (1934) produced a strain maximally resistant to 'Sulphoharnstoff' after 20 passages. Three infected mice were

Table I
Showing the reactions in vivo of the normal and solganal-fast strains of spirochaetes

Drug	D	Normal strain			Resistant strain			
	Dose: mgm. per 20 gm. mouse	No. of mice treated	No. of mice which became negative	Remarks	No. of mice treated	No. of mice which became negative	Remarks	
Solganal	0.25	2	0					
	0.5	3	0					
	0.75	7	1	No action in other mice				
	1.0	7	7					
	4.0				8	3	Infection diminished in 4 other mice	
	6.0			•	7	3	Infection diminished in other mice	
	8:0				3	. 3		
Reduced try- parsamide	0.3	4	1	Infection diminished in 1 other mouse				
	0.5	7	4	Infection diminished in 2 other mice	5	. 4	Infection diminished in other mouse	
	1.0	4	3	Infection diminished in other mouse	4	4		
	2.0	-)	2)		3	3	Toxic	

taken, two were treated intraperitoneally with suitable doses of solganal, and one was kept as a safeguard. On the day following the treatment, one of the treated mice, which still showed spirochaetes in the blood, was killed; its total blood was injected into three more mice, which were treated in the same way; and so on. Occasionally, the sequence of treatments was interrupted for a few passages owing to absence of the writer, but these passages are not included in

the enumeration of the series. The development of solganal-resistance was as follows:

1st passage, 4.11.36: withstood 0.5 mgm., per 20 gm. mouse, intraperitoneally.

12th passage, 14.12.36: withstood 1.0 mgm.

21st passage, 12.2.37: withstood 2.0 mgm.

42nd passage, 11.5.37: withstood 4.0 mgm.

61st passage, 1.7.37: withstood 6.0 mgm.

70th (final) passage, 6.8.37: see Table I.

The reactions of the normal and solganal-fast spirochaetes in vivo are shown in Table I. The mice were treated intraperitoneally on the first day on which spirochaetes were found in appreciable numbers in the blood, which was usually on the second day after inoculation. Since the blood of many mice became free of spirochaetes spontaneously after two to three days, the action of the drug was judged on an examination made 24 hours after treatment. Mice in which the blood became negative following treatment usually relapsed after a few days. From the table it is seen that the minimum effective dose of solganal for the normal strain is 0.75 mgm. solganal per 20 gm. mouse, and of reduced tryparsamide about 0.5-1.0 mgm. The treated strain is almost resistant to a dose of 4.0-6.0 mgm. solganal, but shows no resistance in vivo to reduced tryparsamide. In this case, the development of resistance was much slower than in that recorded by Fischl and Singer (1934), but the reason is obscure. Presumably, if treatment with solganal had been continued, the strain would ultimately have become resistant to the maximum tolerated dose (10 mgm.), but unfortunately the time for continued treatment was not available, owing to a change in the writer's Possibly, if resistance to solganal had been complete, some resistance would also have been found to arsenicals (Fischl and Singer, 1934). Other unpublished attempts by the writer to obtain drug-resistant strains of spirochaetes have shown that this is much more difficult than is the case with trypanosomes. Some years ago at Liverpool a strain of relapsing fever spirochaetes (sp. duttoni) was treated in mice with repeated doses of reduced tryparsamide, but after 50 such passages no increase of resistance could be detected. The same strain was more recently treated with solganal; after 20 passages the minimum effective dose had risen from about 0.25-0.5 mgm. per 20 gm. mouse to about 1.0 mgm.

Spirochaeticidal Action in vitro

The spirochaeticidal action of drugs in vitro was examined by the same technique as that used for their trypanocidal action (Yorke and Murgatroyd, 1930). The medium consisted of 2 parts Locke's solution, containing 0.2 per cent. glucose, and 1 part rabbit serum deactivated by heating to 60° C. for 1 hour. The spirochaetes were obtained from the blood of one or more heavily infected mice, separated by centrifuging, and added in suitable numbers to the

various tubes. The parasites were counted in a wet film under a dark-ground-illumination microscope using a 1/6 objective and 4 ocular, and the number is expressed as living spirochaetes per 20 microscope fields. This is only an approximate enumeration, since the thickness of the wet film may vary considerably, but it is sufficient for the purpose required. The initial number of spirochaetes was usually about 100 per 20 fields, which is equivalent to about 2,000 per mm.³ The parasites in the control tubes usually survived for 24 hours in undiminished numbers under these conditions, but after this they began rapidly to die off. When spirochaetes are killed by the action of a drug, they do not disintegrate so quickly as trypanosomes, but their motionless forms persist for many hours. In these experiments, only the mobile forms have been counted. The protocol of a typical experiment with reduced tryparsamide is given in Table II. It will be noticed that there is a wide range of individual variation in

Table II Showing the action of reduced tryparsamide upon normal spirochaetes in vitro at 37° C.

7D . L	Concentration	No. of living spirochaetes per 20 fields				
Tube	of drug : γ per ml.	Start	After 6 hours	After 24 hours		
1	5	(2	0		
2	$2 \cdot 5$		7	0		
3	1.25		3	0		
4	0.63	100 4	12	0		
5	0.32		100	0		
6	0.16			14		
7	0.08			110		
8	Control	100	120	100		

sensitivity among the spirochaetes, especially during the earlier part of the experiment, and that some of the organisms resist a concentration of drug eight times as great as that which kills the majority. This makes the end point less clearly marked; the minimum spirochaeticidal concentration has been taken as that which is sufficient to kill at least 90 per cent. of the organisms.

The spirochaeticidal action in vitro of a number of different compounds is shown in Table III, which also includes their trypanocidal action; this was measured on the Liverpool strain of T. rhodesiense, mostly in experiments simultaneous with those for the spirochaetes. The minimum concentrations found vary somewhat in different experiments, apparently owing to unknown factors in the rabbit serum used as a culture medium. The values in the table are average figures based on three or more determinations.

Classified according to their parasiticidal action, the compounds fall into four groups.

1. Most of the organic trivalent compounds, viz., reduced tryparsamide, phenyl-arsenoxide, reduced stovarsol, reduced orsanine, reduced neocryl, K.352, novarsenobillon and parosan oxide. All these are active against spirochaetes, and highly active (with the exception of reduced stovarsol and parosan oxide) against trypanosomes. Diamino-methyl-acridine is also fairly active against both.

Table 111
Showing the minimum lethal concentration in vitro at 37° C. of various compounds for normal and solganal-fast strains of spirochaetes and for normal trypanosomes

	Mi	Minimum lethal concentration : γ per ml.					
Drug		Normal spirochaetes		Resistant spirochaetes		osomes	Ratio between trypanocidal and
	Within 6 hours	Within 24 hours	Within 6 hours	Within 24 hours	Within 6 hours	Within 24 hours	spirochaeticidal activity
Reduced tryparsamide .	0.6	0.16	1.2	0.3	0.04	0.008	15-20
FN1 1 1.1	0.13	0.06		-	0.003	0.002	30-40
D 1 1 1	0.5	0.2	***************************************		0.5	0.07	1-3
Reduced orsanine .	0.3	0.16	-	V-PRODUCT-A	0.03	0.007	10-20
Reduced neocryl	0.8	0.16			0.06	0.007	15-20
K.352	0.9	0.2		-	0.03	0.01	20-30
Parosan oxide	$2\cdot 5$	0.3			1.2	$0 \cdot 3$	1-2
P-carboxy-phenyl dichlo	r-						
arsine	7	$2 \cdot 5$	-	******	7	0.8	1-3
Novarsenobillon	0.6	0.3	1.25	0.6	0.08	0.02	8-15
Arsenious oxide	160	80	160	80	1.0	0.3	160-270
Tartar emetic	250	200	250	200	0.5	0.16	500-1,200
Diamino-methyl-acridine	1.25	0.5	1.25	0.5	0.15	0.02	8-25
Solganal	90	50	90	50	500	50	0.2 - 1

2. P-carboxy-phenyl dichlorarsine. This occupies an intermediate position possessing the same low activity on both. It is well known that the presence of a carboxyl group produces a marked diminution of trypanocidal activity, and the same is true, to a relatively smaller extent, of the spirochaeticidal activity.

3. Arsenious oxide and tartar emetic. These inorganic compounds are fairly active against trypanosomes, but are almost completely inactive against spirochaetes.

4. Solganal. *In vitro*, solganal has little action on either type of organism; probably this activity has no connection with the spirochaeticidal action observed *in vivo*, especially as there is no difference *in vitro* between the concentration required for the normal strain and that for the solganal-fast one. Attempts were made to activate solganal *in vitro* by incubation with lysed red blood corpuscles, in a similar manner to the activation of tryparsamide, but this produced a diminution in the activity rather than an increase.

The data given allow a comparison between trypanocidal and spirochaeticidal activity. In the wider range it has long been known that the two are not completely parallel, as is shown by the exclusively trypanocidal action of germanin, and the exclusively spirochaeticidal action of solganal. The present series shows that even in the narrower range of trivalent arsenical compounds the parallelism is not constant. As a general rule, spirochaetes appear to be about 20 times less sensitive to these compounds than trypanosomes; but to reduced stovarsol and parosan oxide they are relatively more sensitive, while to arsenious oxide and tartar emetic they show a surprising resistance. The relatively greater sensitivity to the stovarsol derivative is interesting, since it can be correlated with the therapeutic value of stovarsol against syphilis, which is greater than the unpromising results on murine trypanosomiasis might have led one to expect; similarly, but to a less extent, with novarsenobillon.

Some resistance was shown *in vitro* by the solganal-fast strain, viz., the minimum lethal concentrations of reduced tryparsamide and novarsenobillon were double these for the normal strain, but this was not great enough to allow a more detailed analysis. Since, as described above, the strain was not maximally resistant, no conclusions can be drawn from its behaviour *in vitro*.

Absorption of Reduced Tryparsamide by Spirochaetes

Previous work (Hawking, 1937, 1938a) has shown that the first stage of trypanocidal action is the fixation of the drug by the trypanosomes. Normal trypanosomes will avidly absorb large amounts of compounds such as reduced tryparsamide or acriflavine, when they are exposed to these drugs in vitro or in vivo. The behaviour of spirochaetes was investigated in the same way.

(a) Biological Method. In this technique, heavy suspensions of the organisms are incubated with the drug for some time and are then centrifuged; the presence or absence of the drug in the supernatant fluid is then determined by comparing its trypanocidal activity with that of a series of freshly prepared dilutions of the drug (Hawking, 1937). The tendency of spirochaetes to absorb reduced tryparsamide was examined in this way, low concentrations of drug being used in order that absorption, if it occurred, might more easily be detected. Table IV shows the condensed protocol of a typical experiment. The spirochaetes were counted by making a suitable dilution into Locke's solution containing a few fresh erythrocytes; the proportion of spirochaetes to erythrocytes

TABLE IV

Showing an investigation by the biological method of the absorption of reduced tryparsamide by suspensions of normal spirochaetes

Tube	Initial	Spirochaetes per mm. ³	Activity of supernatant fluid after centrifuging off spirochaetes			
	of drug : γ per ml.		Minimal trypanocidal concentration within 24 hours	Corresponding concentration of drug (tube 4): γ per ml.		
1	0.08	$2 \cdot 1 imes 10^6$	1:8	0.08		
2	0 (control)	$2 \cdot 1 \times 10^6$	1:2			
3	0.08	0 (control)	1:8	0.08		
4	0.08 (standard)	downed	1:8			

Volume per tube, 1 ml. Tubes 1-3 were incubated for 1 hour at 37° C. and then centrifuged; tube 4 (standard) was freshly mixed and not incubated or centrifuged.

was determined on the dark-ground-illumination microscope, and the number of erythrocytes was then counted in an ordinary haemocytometer. Tube IV contained a solution of drug in culture medium, freshly prepared at the last moment without incubating or centrifuging; and this served as a standard for

TABLE V

Showing an investigation by the chemical method of the absorption of reduced tryparsamide by suspensions of normal and solganal-resistant spirochaetes

Tube	Initial concentration	R.B.C.	Spiroch	As ₂ O ₃ in deposit :	
	of drug : γ per ml.	per ml.	Start	After 65 min.	γ
l	20	2,600	290,000/mm. ³ Normal	Active	1.5
2	20	1,900	720,000/mm. ³ Resistant	Active	•)
3	20	26,000	0 (control)		0

Volume per tube, 5 ml. Total arsenic-content per tube, $40 \gamma \text{ As}_2\text{O}_3$. Tubes incubated at 37°C . for 65 minutes, and then centrifuged; deposits washed once with 5 ml. fluid.

the trypanocidal activity of the supernatant fluids from the other three tubes. The table shows that no tendency of heavy suspensions of spirochaetes to remove reduced tryparsamide from the surrounding fluid can be detected by this method.

(b) Chemical Method. In this technique, the organisms are incubated with the drug in vitro, centrifuged, and washed; their content of arsenic is then determined chemically by the method of Monier Williams, using a lead electrode (Hawking, 1937). This method is less delicate than the biological one, requiring the use of much larger volumes, but it is more satisfactory for investigating cases in which only a small portion of the total compound is absorbed. The protocol of a typical experiment is shown in Table V. The medium used consisted of one part serum and five parts Locke solution containing glucose. It is practically impossible to obtain large numbers of spirochaetes completely free from red blood corpuscles. Consequently a count was made of those present with the organisms; a control tube (tube 3) was set up containing a larger number of corpuscles; and the arsenic-content of the eventual deposit was estimated in the same way as those from the tubes containing spirochaetes. As is seen, the amount of arsenic absorbed by this number of corpuscles is inappreciable, and the absorption in tubes 1 and 2 must therefore be due to the spirochaetes present. The table shows, therefore, that, when exposed to relatively strong concentrations of reduced tryparsamide, spirochaetes do absorb a small quantity of the compound. The amount absorbed is smaller than that absorbed by normal trypanosomes under the same conditions, even if allowance be made for the smaller bulk of the spirochaetes. The amount per million organisms absorbed by the resistant spirochaetes is less than that absorbed by the normal ones, but little significance can be attached to this, in view of the low accuracy of microestimations of arsenic. Other experiments gave similar results.

Photosensitivity

When trypanosomes are exposed to acriflavine, they become photosensitive, so that when placed in a strong light, as on the stage of a dark-ground-illumination microscope, they rapidly become motionless and presumably dead. The degree of photosensitivity can be measured as the duration of illumination in seconds required to produce immobilization, which for convenience has been designated the 'light-index'. Spirochaetes were studied by the same technique (Hawking, 1938b), being exposed *in vitro* to diamino-methyl-acridine at 37° C. The results are shown graphically in the accompanying figure, in which the logarithm of the concentration of the drug is plotted against the logarithm of the light-index.

For convenience, the logarithm of the 'photosensitivity' is also shown, the 'photosensitivity' being taken as 100/light-index. As is seen, there is no appreciable difference between the behaviour of the normal and of the solganal-fast strains (possibly owing to the incomplete character of the solganal-resistance).

Allowing for experimental variation, the points approximate to a straight line (drawn freehand) corresponding to the equation

$$0.87 \log C = 0.85 - \log T$$

i.e., $C^{0.87} T = 7.08$,

where C is the concentration of the compound, γ per ml., and T the light-index in seconds. This is similar to the expression found in a previous paper (Hawking, 1938b) for normal trypanosomes exposed to acriflavine, viz.,

$$0.86 \log C = 1.97 - \log T$$
.

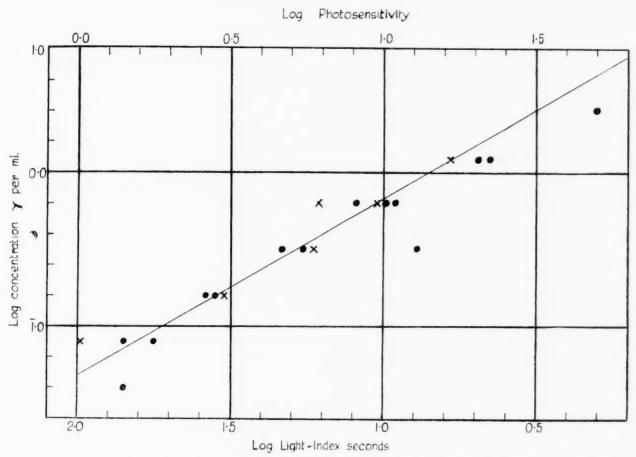


FIGURE showing the relation between the photosensitivity of spirochaetes and the concentration of diamino-methyl-acridine.

Spots refer to normal spirochaetes and crosses to solganal-fast ones. Photosensitivity 100/light-index.

DISCUSSION

The main interest of these investigations lies in the light which they may throw on the probable similarities and differences in the chemotherapeutic behaviour of trypanosomes (on which most of the experimental work is done) and of *Treponema pallidum* (to which most of the clinical attention in temperate zones is directed). Although relapsing fever spirochaetes are not identical with those of syphilis, they probably resemble them more closely than trypanosomes

do. The parallelism between trypanocidal and spirochaeticidal action has been discussed above in connection with Table III; it would seem well worth while, in examining new compounds, to supplement the tests on trypanosomes with tests on relapsing fever spirochaetes.

In its sensitivity to the trivalent phenyl-arsenical compounds, the present strain of spirochaetes seems to lie midway between the normal and the atoxyl-fast strains of T. rhodesiense, the minimum lethal concentration (within 24 hours) of reduced tryparsamide being approximately $0.008 \, \gamma$ per ml. for the normal trypanosomes, 0.16γ per ml. for the spirochaetes, and 5γ per ml. for the resistant trypanosomes. This lower sensitivity of the spirochaetes, compared with that of the normal trypanosomes, can be correlated with the lower tendency of the spirochaetes to absorb the arsenical from the surrounding fluid. The average of four experiments like that of Table V showed that 5×10^9 spirochaetes absorbed about 7 γ reduced tryparsamide when exposed to a concentration of $20 \, \gamma$ per ml. The volume of spirochaetes is difficult to estimate, since they are hard to separate completely from platelets and other débris; but experimental determination gave the volume of 109 spirochaetes as about 5 mm.³ Consequently, the concentration in the deposit of spirochaetes would be of the order of 300 γ per ml., and the ratio (partition-ratio) between the internal and external concentrations would be 15. For normal trypanosomes the partition-ratio of reduced tryparsamide has been calculated as about 5,000, and for resistant trypanosomes as about 5 (Hawking, 1938a).

The literature concerning the relation between drug-resistance in spirochaetes and their absorption or non-absorption of the corresponding compounds has been reviewed in a previous paper (Hawking, 1937). The resistance of the present solganal-fast strain was incomplete, and it is therefore not possible to draw general conclusions from its behaviour; but the work described above shows that even normal spirochaetes possess only a relatively low tendency to absorb arsenical compounds, and that consequently the differences, if any, observed between the two strains will be correspondingly restricted in extent.

SUMMARY

- 1. The chemotherapeutic reactions of relapsing fever spirochaetes have been examined *in vitro* by the methods previously used for trypanosomes.
- 2. There is an approximate parallelism between the activity of a series of organic trivalent arsenic compounds and of diamino-methyl-acridine upon spirochaetes and upon trypanosomes (*T. rhodesiense*), the minimum lethal concentration *in vitro* for the spirochaetes being about 20 times as great as that for the trypanosomes; but the spirochaetes are relatively more sensitive to reduced stovarsol and to parosan oxide, and they are insensitive to sodium arsenite and to tartar emetic.
- 3. Solganal has little action upon spirochaetes or trypanosomes in vitro, and it cannot be activated by the addition of lysed red blood corpuscles,

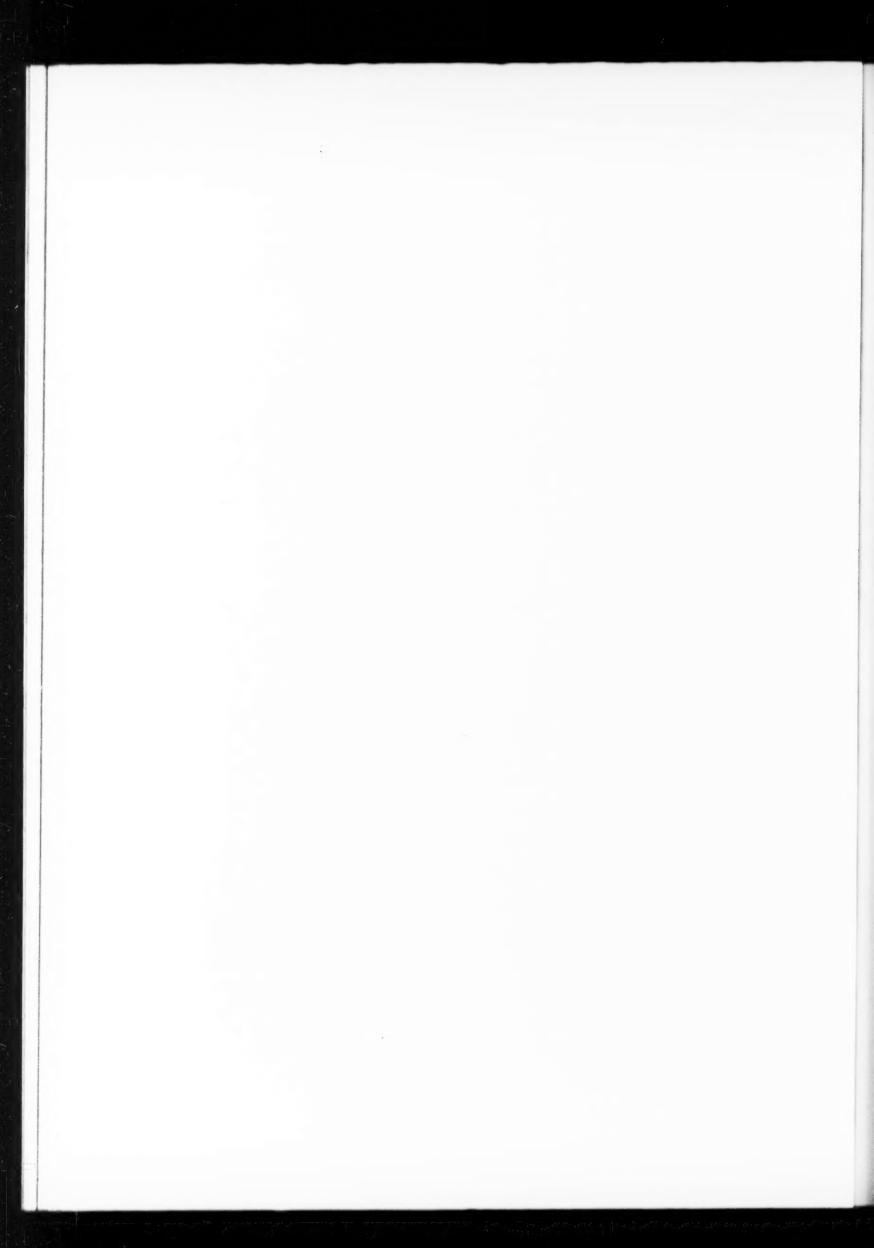
- 4. Spirochaetes tend to absorb organic trivalent arsenical compounds when exposed to them *in vitro*, but the amount absorbed is very much less than is the case with trypanosomes.
- 5. Spirochaetes exposed to diamino-methyl-acridine become photosensitive.
- 6. By repeated treatment with solganal *in vivo*, a solganal-fast strain could be obtained, but its development was very slow, and even after 70 passages resistance was still incomplete.

ACKNOWLEDGEMENTS.—Acknowledgements are due to Professor H. Schlossberger for the strain of spirochaetes, and to Dr. H. King and Dr. A. J. Ewins for many of the compounds. This work was assisted by a grant from the Medical Research Council.

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CONTRIBUTION ON THE MODE OF ACTION OF GERMANIN (BAYER 205)

BY

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(Received for publication July 26th, 1938)

The purpose of this paper is to describe experiments conducted *in vitro* upon the trypanocidal action of germanin (Bayer 205), the object being to compare and contrast the behaviour of this compound towards trypanosomes with that of other compounds (trivalent arsenicals and acriflavine) studied in previous papers (Hawking, 1937, 1938).

The trypanosomes were obtained from the strain of *T. rhodesiense*, kindly supplied by Professor Warrington Yorke, which has been used in previous work. The germanin was kindly provided by Bayer Products, Ltd.

Production of Germanin-Resistant Strain

As a preliminary measure, a strain of trypanosomes was made germanin-As is well known, the production of a strain resistant to this compound by the usual method is a slow and tedious procedure; so a special technique, described by Jancsó and Jancsó (1935) was adopted, using animals in which the reticulo-endothelial system has been blockaded as far as is possible. In the present instance, rats were used which had been splenectomized several days before infection. Shortly before the first dose of germanin, they were given intravenously about 1 ml. per 100 gm. of electro-colloidal copper solution (obtained from Messrs. von Heyden, Dresden), and this was repeated after 2-3 weeks if the animal survived. The germanin was injected intraperitoneally in doses sufficient temporarily to diminish the number of trypanosomes in the blood. The minimum effective dose for this strain is somewhat less than 0.2 mgm. per 100 gm. rat. Treatment was commenced on March 1st, 1937. Resistance developed steadily, until by July 9th, 1937, repeated doses of 30 and 40 mgm. per 100 gm. failed to sterilize the blood of blockaded rats, and treatment was discontinued. In normal rats, it was found that the strain resisted doses of 5-10 mgm., but not 20 mgm. The development of resistance by this technique is much more rapid than is the case when unblockaded animals are used. In the present instance, resistance was almost maximal after four months; on a previous occasion (Yorke, Murgatroyd and Hawking, 1932), when normal mice were used, 17 months were required. At several stages of the treatment, blood-smears were made from rats on the day following a dose

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of germanin; numerous abnormal dividing forms with three or four nuclei were seen, similar to these described by Jancsó and Jancsó (1934). The use of these blockaded rats was seriously incommoded by the fact that after splenectomy a large proportion of them developed severe anaemia, accompanied by *Bartonella* in the red blood corpuscles, which was often fatal.

Trypanocidal Action of Germanin in vitro

This was examined by the usual technique (Yorke and Murgatroyd, 1930). The minimum trypanocidal concentration at 37° C. after 6 hours' exposure was 5 mgm. per ml.; after 24 hours' exposure 0.31 mgm. per ml. killed the majority of organisms, although 1.25 mgm. was required to kill 100 per cent. The behaviour of the two strains, normal and resistant, was identical in this respect.

Table I
Showing the effect of exposing normal trypanosomes to germanin in vitro upon their power of infecting mice; duration of exposure required

713 1	Concentration	Period of	Trypanosomes per mm.3		Example in the last
Tube	of drug : mgm. per ml.	exposure : hours	Start	After washing	Fate of mice inoculated
ı	0.1	1.3	1,600	1,200	Infected; 5 days' incubation
2	0.1	2	1,600	1,100	Not infected
3	0.1	3.2	1,600	1,600	Not infected
4	0.1	4.5	1,600	1,000	Not infected
5	0 (control)	4.5	1,600	800	Infected; 2 days' incubation

Temperature, 37° C. Two mice inoculated intraperitoneally from each tube with 0.45 ml. per mouse.

Allowing for the variation which occurs in these experiments, the findings are in agreement with those given by Yorke, Murgatroyd and Hawking (1931a) for the minimum trypanocidal concentration of germanin after 24 hours' exposure, viz., 0.62 mgm. per ml., although considerably greater than those given by Jancsó and Jancsó (1934), viz., about 0.05 mgm. per ml. after 24 hours. In any case, since this trypanocidal action in vitro is the same for both normal and resistant trypanosomes, it is probably unconnected with the trypanocidal action observed in vivo.

Disarming Action of Germanin in vitro

Normal trypanosomes were incubated in culture medium (1 part serum plus 2 parts Locke's solution with 0.2 per cent. glucose) containing 0.1 mgm. germanin per ml., a concentration much below that required to kill the organisms

in vitro. After various intervals the trypanosomes were centrifuged down, washed with an equal volume of medium, centrifuged again and resuspended in an equal volume of fresh medium; they were then counted, to control losses due to manipulation, and finally inoculated intraperitoneally into mice. In all cases, the organisms were actively motile shortly before inoculation. The results of a typical experiment are given in Table I, which shows that mice inoculated with trypanosomes which had been exposed to this concentration of germanin for 2 hours or more did not become infected (period of observation 21 days), those inoculated with trypanosomes exposed for 1·3 hours become infected after 5 days, and those inoculated with trypanosomes from the control tube become infected after 2 days. The failure of trypanosomes from tubes

Table II

Showing the effect of exposing normal and resistant trypanosomes to germanin in vitro upon their power of infecting mice; concentration of germanin required

Tube	Strain of	Concentration of germanin: mgm. per ml.	Trypanosomes per mm. ³		Fate of mice inoculated
	trypanosomes	nigin. per im.	Start	After washing	
I	Normal	0.1	1,900	830	Not infected
2	.,	0.01	1,900	1,300	Infected; 4 and 5 days' incubation
3	,,	0.001	1,900	960	Infected; 2 days' incubation
4	Normal	0 (control)	1,900	1,000	Infected; 2 days' incubation
5	Resistant	1.0	2,500	2,500	Infected; 2 days' incubation

Temperature, 37° C. Duration of exposure, 3 hours. Two mice inoculated intraperitoneally trom each tube with 0.4 ml. per mouse.

2, 3 and 4 to infect mice cannot be due to small quantities of germanin carried over in the inoculation fluid, since similar quantities would have been carried over with the trypanosomes of tube 1. In some way, germanin must sensitize the trypanosomes to the defence mechanisms of the body, so that, although they appear normal and their motility is unimpaired, when inoculated into susceptible animals they fail to infect them. This phenomenon, which has also been observed by Nauck (1925), Reiner and Köveskuty (1927) and Issekutz (1933), is in striking contrast to our experience with trivalent arsenicals under similar circumstances (Yorke, Murgatroyd and Hawking, 1932); when trypanosomes are exposed *in vitro* to reduced tryparsamide or halarsol, until all but a very few organisms are killed, and then washed and inoculated into mice, infection nearly always results.

Experiments were performed to ascertain the minimum concentration of germanin required to produce this disarming effect, and a typical protocol is reproduced in Table II, which also includes a simultaneous experiment upon the germanin-fast strain. The table shows that under these conditions the minimum concentration required for the normal strain is about 0·1 mgm. per ml.; concentrations of 0·01 mgm. per ml. occasionally were sufficient to prevent infection, but this was not usually the case. It must be remembered that absence of infection depends upon the disarming of 100 per cent. of the organisms; if even a few escape the influence of the drug they will be capable of producing infection; probably concentrations much below 0·1 mgm. would be sufficient for the majority of the organisms. In striking contrast to the normal trypanosomes, the germanin-fast trypanosomes are not appreciably affected even by concentrations of 1·0 mgm. per ml. This difference between the two strains, similar to that observed in vivo, confirms the significance of the phenomenon here studied.

Absorption of Germanin in vitro

In previous papers (Hawking, 1937, 1938) experiments have been described on the absorption of arsenicals and acriflavine by trypanosomes *in vitro*. Analogous experiments were performed with germanin, using a chemical method of estimation described by Dangerfield, Gaunt and Wormall (1938), whose paper should be consulted for full details.

Technique

Briefly the method is as follows. About 2 ml. of the suspected fluid is boiled for 6 hours with 3 ml. concentrated hydrochloric acid. It is made up to 10 ml., and decolorized with kaolin. Two ml. of the filtrate is treated successively with 1 drop 0.5 per cent. sodium nitrite, 2 drops saturated urea, 3 ml. 30 per cent. sodium acetate, and 1 ml. 0.5 per cent. mono-methyl-a-naphthylamine in 50 per cent. acetic acid. A red colour develops which is matched colorimetrically against known solutions of germanin treated in the same way. By this technique, quantities down to 0.01 mgm. can be detected.

Heavy suspensions of trypanosomes were made in a medium consisting of 1 part serum and 5 parts Locke-glucose solution. They were incubated for about 1 hour at 37° C., after which they were examined microscopically. They were then centrifuged and washed once with an equal volume of flesh fluid; the germanin-content of the final deposit was determined as above. In the earlier experiments, the germanin-content of the supernatant fluid was also determined, but, as no significant change was usually found, this was subsequently omitted. Trypanosomes do not survive so well if they are present in large numbers, and in some of the experiments it was found that practically all the organisms were dead at the end of the period of exposure. When the organisms were living at the end of the exposure, it was found that only small amounts of germanin had

been absorbed. When the organisms were all dead, the deposit contained large amounts of the compound. Table III shows the protocols of two typical experiments, performed on different days, in which the trypanosomes survived or died during the exposure, respectively. The amount of germanin absorbed by the living trypanosomes is so small that the amount can be estimated only approximately. Similar amounts were found when trypanosomes were exposed to concentrations of one-tenth this concentration. This probably represents the amount required to saturate living organisms, independent (within limits) of the external concentration. The amount absorbed by dead trypanosomes is

Table III Showing the absorption of germanin by normal and resistant trypanosomes when exposed to this compound in vitro at 37° C.

713 1	Initial concentration of germanin: mgm.	Duration of exposure : minutes	Trypanosomes		Germanin: mgm.	
Tube			Start	End of exposure	In trypanosomes	In supernatan fluid
		A. A	Absorption by living	ing trypanosom	es	
1	1.0	40	106,000/mm. ³ Normal	Feebly motile	0.01	10
2	1.0	75	102,000/mm. ³ Resistant	Part motile, part dead	0.04	
	A STATE OF THE PARTY OF T	В.	Absorption by de	ad trypanosome	es	
3	1.0	60	640,000/mm. ³ Normal	All dead	0.69	
4	1.0	60	210,000/mm. ³ Resistant	All dead	0.44	

Volume per tube, 10 ml.

about 10 times as great (allowing for the difference in numbers). Dead cells often absorb more readily than living ones, and in previous papers it was shown that the absorption of arsenicals and of acriflavine by resistant trypanosomes was much increased by death. This increased absorption has obviously little significance in connection with the therapeutic action. Apparently, even with dead trypanosomes, absorption of germanin is a slow process (contrast the absorption of arsenicals and acriflavine), since even in tubes 1 and 2, in which the amount taken up was small, many of the trypanosomes were dead.

Allowing for the different numbers present, no evidence was obtained during the course of these experiments that resistant trypanosomes had less tendency than normal trypanosomes to absorb germanin. The present finding—that living trypanosomes have little tendency to absorb germanin in vitro—is in agreement with the result obtained by Issekutz (1933), who found only small amounts of the compound in trypanosomes which had been exposed to it in vivo.

DISCUSSION

Further work on the subject was interrupted by a change of appointment, so that elucidation of the points raised was not possible. The results, however, are sufficient to show the striking contrast between germanin and the trivalent arsenicals in vitro, parallel to that which has long been known to occur in vivo. Thus, germanin has no significant trypanocidal action in vitro and is absorbed by trypanosomes only to a small extent. Trivalent arsenicals and acriflavine have a powerful trypanocidal action in vitro and are absorbed in very large amounts. Trypanosomes which have been exposed to germanin in vitro, and which have presumably absorbed small quantities of the compound, will continue to live for over 24 hours in vitro but fail to infect mice into which they are inoculated. Trypanosomes exposed to arsenicals or acriflavine under the same conditions will infect mice but will not live in vitro. These facts are in agreement with the explanation of Reiner and Köveskuty (1927) and of Jancsó and Jancsó (1934), viz., that germanin has an opsonin-like effect, sensitizing the trypanosomes to phagocytosis by the reticulo-endothelial system. It is hoped to return to these investigations at some later date.

SUMMARY

- 1. A strain of trypanosomes was made resistant to germanin, using Jancsó's technique, involving animals in which the reticulo-endothelial system had been blockaded.
- 2. Germanin has practically no trypanocidal action *in vitro*; but if normal trypanosomes are incubated with it *in vitro*, and then washed and inoculated into mice, they fail to infect. Germanin-fast trypanosomes do not lose their infectivity under these conditions.
- 3. When living trypanosomes are exposed to germanin *in vitro* the amount absorbed by the organisms, as judged by chemical estimation, is small; no difference was detected between normal and resistant trypanosomes in this respect. Dead trypanosomes absorb the compound more readily.

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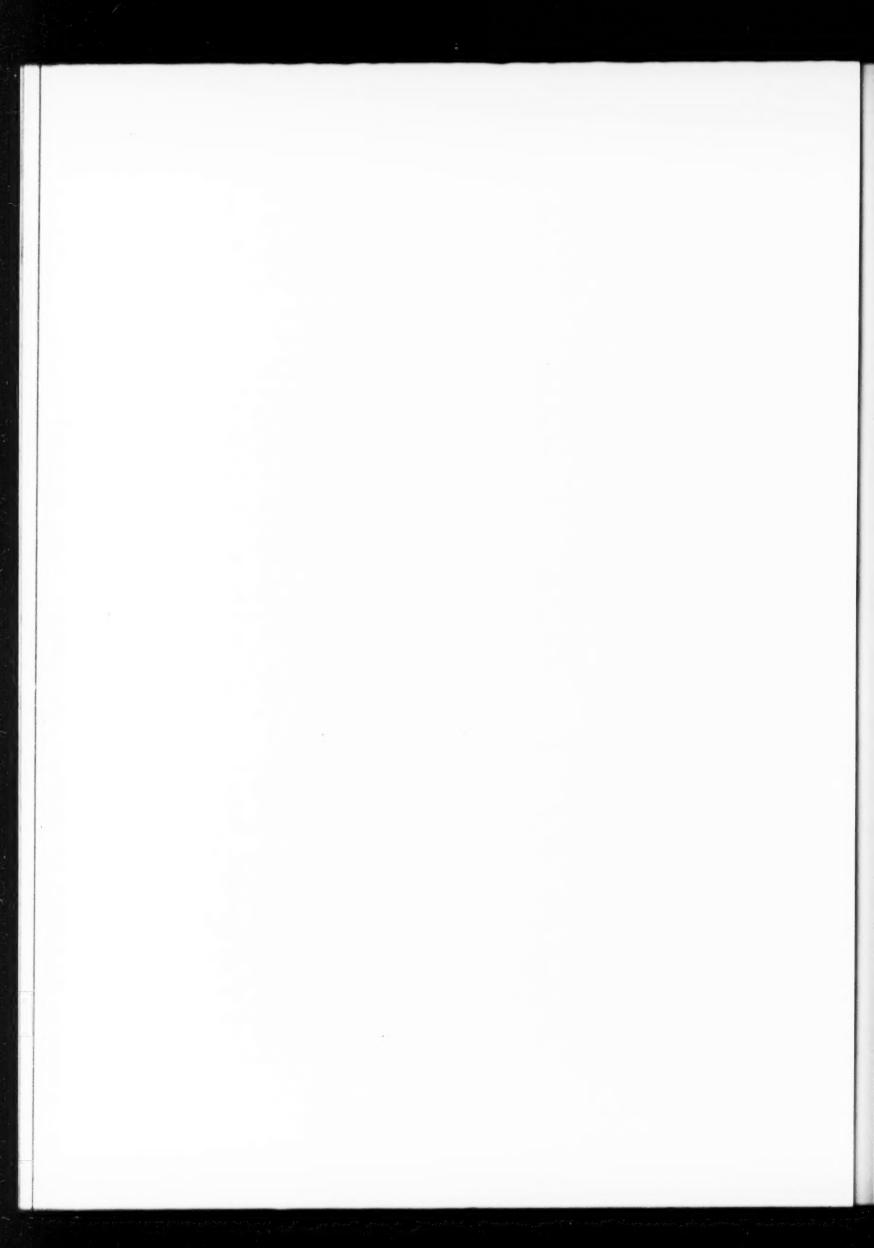
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PARALYSIS AS A CLINICAL MANIFESTATION IN HUMAN RABIES

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To the lay mind, and at times also to the medical mind, rabies is pictured as a disease in which the prominent feature is excitement or 'fury'. The names, indigenous and imported, by which the condition is known in various countries may be shown to be derived from a root-word indicating madness. Even the inappropriate term 'hydrophobia' suggests excitement and fear. conception of the disease in man, paralysis has not appeared as a distinguishing or characteristic symptom, nor has it been regarded hitherto as a sign of sufficient importance to justify its consideration in the differential diagnosis of rabies. In animals, as is well known, the disease may manifest itself either in a furious form (la rage vrai) or in a paralytic form (la rage mue), but even here the paralytic feature occupies the background in our minds. In the case of the human disease, there are some who, according to Muir and Ritchie (1927), deny the existence of paralytic rabies in the sense that paralysis can constitute the main and primary manifestation. The classical description of the disease as it affects man recognizes three stages, viz., a prodromal stage, one of excitement, and a short terminal stage of paralysis followed by death. This paralysis is apparently consequent on the exhaustion due to the irritation and over-stimulation of the nerve-centres during the second stage. It is during this second stage that clinical symptoms arise which, apart from the history of a bite, may permit a diagnosis of rabies to be made. Paralysis as a sign antecedent to, or independent of, the stage of nervous excitement and irritability was therefore not known to be a feature of human rabies.

Following Pasteur's introduction in 1885 of prophylactic vaccination against the disease by the use of tissue from the central nervous system of rabbits, instances of paralysis after bites by rabid animals and after such treatment had begun were noticed in various countries and have continued to occur to the present day. Remlinger (1928) states that, of 1,164,264 persons so far treated, 329 have developed paralysis. The paralytic manifestation is definite and distinct, and may appear as a result either of a spreading myelitis like a Landry's paralysis, or of only a localized dorso-lumbar involvement. Recoveries from both forms have been observed. Various explanations have been offered to account for this paralysis. Some are of opinion that it is due to a toxin or ferment engendered in the vaccine during its preparation, or to a modification of the original infection by the superadded vaccination. Others, again, maintain that the paralysis is produced by some toxic substance which is to be found in

normal brain or cord material, though the observations of Hurst (1932) suggest some additional factor. Whatever may be the true explanation, it is now established that it is not due to, or associated with, the living virus of rabies. Such cases of paralysis following antirabies treatment, as well as those cases of rabies developing paralysis subsequent to the second stage of irritation, are not therefore instances of paralytic rabies, and should clearly be distinguished from the cases

and material which form the subject-matter of this paper.

Though paralysis was not generally regarded as a characteristic sign in human rabies, the literature available locally shows that there were voices crying in the wilderness, directing attention to its occurrence. Park and Williams (1934) stated that in 1870(?) Van Sweiten observed the paralytic form of rabies in human beings. In the report dated June, 1887, submitted by the committee consisting of Lister, Horsley, Brunton and others after their visit to Paris to investigate Pasteur's treatment, reference is made to a man named Goffi who was bitten in London, and, in spite of treatment, succumbed to an acute ascending The inoculation of his spinal cord into rabbits produced typical paralytic rabies. The following interesting observation was made in that report. 'To the last Goffi was free from all the usual symptoms of hydrophobia, and the progress of his disease, and the manner of his death, were so similar to those of what is described as acute ascending paralysis, or Landry's paralysis, that a verdict to this effect was given at a Coroner's inquest'. The further important statement was then added that 'cases of paralytic hydrophobia have been observed, though rarely, in men bitten by rabid animals, and not treated by inoculation. It may, indeed, be suspected that at least some of the cases of acute ascending paralysis may have been cases of this form of hydrophobia, although, in the complete absence of the usual violent symptoms, no suspicion of the source of the disease was entertained'. In his description of the clinical symptoms of rabies, Sir William Gowers (1888), referring to this same man Goffi, made the independent observation that 'the only example of a true paralytic form of rabies hitherto observed in man is that of a patient Goffi. The symptoms closely resembled those of acute ascending paralysis. This case suggests that some other instances of this mysterious form of palsy may have been the result of rabies—a possibility that should be kept in mind by those who meet with examples of this paralysis'. These isolated observations did not receive confirmation or attract attention in England, even in the days when rabies occurred in man and animals, while in France and Germany the opinion prevailed that paralytic rabies never developed in man but was to be found only in rabbits, and occasionally in dogs. It was Gamaleia (1887), in Odessa, South Russia, who, from a valuable study of a series of cases coming within his own experience, and from the recorded observations of others, questioned Brouardel's (1887) classical description, and definitely established that paralysis may appear independently of the second stage of excitement, and may be the most prominent clinical manifestation in human rabies. Gamaleia was of opinion that a nervous

constitutional diathesis in the person bitten, together with the entrance of a large quantity of virus, provoked the paralytic form of the disease instead of the usual furious form, as it was observed that in most cases the wounds were multiple and deep. In one instance, 8 out of 17 persons bitten severely by a rabid wolf developed rabies, and, of these 8, 4 died from the paralytic form of the disease. The cases studied and described by Gamaleia fell into two groups: one in which the paralysis began in the limb bitten (10 such cases were recorded); the other, consisting of 7 cases, showed the paralysis beginning in a limb other than the one bitten. There were, in addition, 10 other cases, of which details were not given. Gamaleia reported on a third group, which conformed to the classical manifestations of the disease where paralysis follows the stage of irritation and excitement. This group will not, of course, be considered here. In every instance the history of a bite by an animal was obtained. The animals incriminated were the dog, cat, fox, wolf and squirrel. Clinically the cases were in many respects similar. There was the development of subjective symptoms of paraesthesia, then the appearance of a paralysis, which was preceded in some cases by paresis. The paralysis did not spread in a regular ascending manner, but jumped from one area or limb to another. There was no recovery when once symptoms developed. In seven of the cases of the first group there was difficulty in swallowing, and in the second group it was present in six. The diagnosis of rabies would have been impossible if based solely on the appearance of paralysis and in the absence of the history of a bite by a rabid animal. Gamaleia drew attention to the fact that, while it was admitted that there was much uncertainty and obscurity with regard to the pathology of rabies, there was also need for modification of the prevalent conception of its symptomatology, so as to include the paralytic form of the disease. Le Gendre (1887) immediately recognized the importance of Gamaleia's observations, and pointed out that paralytic rabies does occur in man as a definite and separate clinical syndrome. He agreed with the opinion that a large quantity of virus, together with an enhanced receptivity on the part of the victim, leads to the development of the paralytic disease. The fact that in rabbits, which are the animals most susceptible to rabies, the disease manifests itself almost invariably as a paralysis lends support to this view. Gamaleia's work did not receive further notice until Bamberger (1896) described two cases of paralytic rabies occurring in human beings in Austria, in which, despite the most careful interrogation of the patients and their relatives, no history of a bite or contact with a rabid animal could be elicited. The first case showed typical symptoms of irritation and excitement with hydrophobia—symptoms which were sufficient to justify the diagnosis of rabies. On the 4th day of illness, and 20 hours before death, while secondary symptoms continued, the patient showed a paresis of his left upper extremity and an inability to move the shoulder and elbow, then a paresis of his right arm and of the back muscles. Post-mortem examination confirmed the diagnosis of rabies. The other case showed as the first sign of illness a paresis of his right

arm and leg, and to a lesser degree of the left leg, after which other typical symptoms of rabies developed, followed by death. Bamberger drew attention to Gamaleia's observations, and agreed with the opinion that a paralytic form of rabies in the true sense does occur in man. These observations received no support (except from Remlinger, as mentioned below), until Marie and Chatelin (1919) gave an account of a child who received a superficial bite on the lip from a rabid dog, and, after a series of 'fits' and 'psychic symptoms', developed six weeks later a complete flaccid paralysis of both lower extremities, with a paresis and paralysis of the upper, followed by death. Difficulty in swallowing was present, and the case was classified as one of paralytic rabies with the development of paralysis elsewhere than in the area bitten. Marie and Chatelin pointed out that rabies may develop as an acute ascending poliomyelitis or as an ascending Landry's paralysis, and remarked that Gamaleia's suggestion to the effect that a heavy infection produces paralytic rabies was not tenable in this case, as the wound on the lip was a slight one. In their paper, reference is made to a similar case of paralytic rabies recorded by Lesieur and others. These cases have all been reported from Europe; but Uribe (1924), in Colombia, South America, described a case of paralytic rabies which assumed the form of an ascending paralysis. The patient, a child, was bitten by a rabid cat on the right leg, and 15 days later developed a paresis and then a flaccid paralysis of both lower extremities. The paralysis spread to the abdomen and the upper extremities, dysphagia with abundant salivation followed, and death took place nine days after the appearance of the first symptoms. The paralysis developed before any other sign and was the most prominent feature throughout the illness. Uribe saw in this case of rabies the symptomatology of an acute ascending poliomyelitis or of a Landry's paralysis. Joseph Koch (1930) devoted much space in his article on rabies to a description of a paralytic form of the disease. He pointed out that the condition was less infrequent in dogs, and was known to Hoin, Laufenauer, Hogyes and Gamaleia. He estimated the proportion of 'furious' rabies to paralytic as 4 to 1, and stated that of 90 deaths at the Pasteur Institute, Paris, from 1886 to 1889, 30 were due to the paralytic disease. Clinically, he distinguished four types of paralytic rabies: one which develops as an acute ascending myelitis or Landry's paralysis; another which appears as an acute paraplegia, with paralysis of the bladder and rectum and just a mild paresis of the upper extremities; a third which produces only a facial paralysis, which may persist for days or weeks; and a fourth which shows itself as a neuritis, in which irritation of the peripheral nerves, due to localization of the virus, is the main and only evidence of disease. Koch is an advocate of the existence of abortive and mild forms of rabies, and maintains that recovery from rabies does occur in man. He is of the school which holds that paralysis with recovery after Pasteur's treatment represents an abortive form of infection. His clinical description and statistical analysis are therefore based on forms of paralytic rabies which may not be accepted by others as cases of rabies, especially in the absence of material for pathological examination due to recovery of the patients. Koch's experience was gathered in Germany. On the other hand, Kraus, Gerlach and Schweinburg (1926), who worked in Austria, stated that in several years' observation they had never seen a single case of paralytic rabies in man, though they admitted that others had noted the condition, especially Babes and Gamaleia. But no mention is made by them, or by the other authors here quoted, to the very valuable work of Remlinger (1906, 1932). In his study of acute ascending paralysis, Remlinger showed that the condition described in 1895 by Octave Landry, and since then bearing the name of 'Landry's paralysis', was not a disease entity, but a clinical syndrome due to a multiplicity of causes. From an investigation of 10 cases Landry described a peculiar form of acute flaccid paralysis (une paralysie centripète) starting in the lower extremities and spreading to the trunk and the upper limbs, to involve the respiratory muscles and centres, and leading to death within a few hours or days-though recovery was known to occur. Post-mortem examination revealed no characteristic naked-eve appearance, and with the methods then available no specific lesion could be detected microscopically. Landry's classical description rescued this symptom-complex from a variety of other paralyses, and has received approval from every direction. In the words of Widal and Sourd, 'la maladie de Landry est restée inattaquable dans son expression symptomatique'. But it was left to Remlinger to show definitely that Landry's paralysis is not an anatomical, aetiological or pathological entity, but a syndrome referable to a variety of agents. It may be due to affections of the peripheral nerves or to primary lesions of the spinal cord, and may be produced by lead, arsenic and other intoxications, or by microbic infections with the pneumococcus, streptococcus, B. diphtheriae, etc., or by syphilis, and the virus of poliomyelitis and rabies, while, on the other hand, there is a group for which no known cause, bacterial or otherwise, can be discovered (le syndrome de Landry primitif). With the progress of time and the detection of the aetiological agents, the number of cases in this group is gradually Landry's paralysis is especially frequent in certain epidemics of 'infantile' poliomyelitis, and has been reproduced experimentally in monkeys by artificial inoculation with the infecting virus. It was Remlinger, however, who emphasized the fact that there was a form of acute ascending, or Landry's, paralysis due to the virus of rabies, which occurs in human beings and in animals under both natural and artificial conditions. Gamaleia, as already mentioned, had previously pointed out that paralytic forms of rabies do develop in man, but in the majority of the cases recorded by him the paralysis involved the limbs in an irregular erratic fashion, suggesting implication of the peripheral nerves, or of disseminated, isolated areas of the spinal cord. The paralysis did not spread in that definite ascending or descending manner which is so characteristic of Landry's, though, as argued by Collier, Montel and others, a polyneuritis which leads to paralysis of a limb must necessarily affect a corresponding portion of the cord. Such an involvement cannot, however, be regarded as a solid,

spreading myelitis. From his experience, Remlinger maintained that there was a paralytic form of rabies in which paralysis begins in the lower extremities, spreads to the bladder and rectum, and then to the upper limbs and the medulla, exhibiting the typical Landry's syndrome due to an acute spreading myelitis. The striking symptom of hydrophobia, which has been regarded as pathognomonic of rabies, may be absent, or may be reduced to a few terminal spasms of the pharynx. Further, difficulty in swallowing may occur in forms of acute ascending paralysis not due to rabies. Such cases of paralytic rabies were, Remlinger showed, familiar to Babes, Hogyes and Van Gehuchten. Remlinger was also of the opinion that instances of paralytic rabies are not as infrequent as statistics would indicate, and it is consequently imperative in all cases of acute ascending paralysis, and other paralyses without obvious pathological cause or lesion, to inquire into a possible history of a bite or contact with a rabid animal, and to undertake experimental and histological examination of post-mortem material, as suggested 45 years previously by the English workers. Such a procedure, he maintained, would detect hidden cases of paralytic rabies. But it should be noted that the absence of such a history does not prove the case to be not one of rabies, as shown by the experiences of Bamberger (1896), Glusmann (1928) and Knutti (1929). Glusmann's case in Poland was not of the paralytic type, but, as described above, Bamberger's was. Knutti's case in the United States is so remarkable, and so similar to the Trinidad cases of paralytic rabies, that an account of it is necessary. A female, age 23, without a history of a bite or contact with a rabid animal, developed a vague pain in her left leg, followed two days later by paralysis and loss of sensation in the whole of that leg and thigh. In another two days there appeared a paresis of the right foot, with incontinence of urine, and, on the following day, a complete paraplegia, which was succeeded by numbness in both hands, abdominal distension, cyanosis, gasping respiration, and death five days after the appearance of the first symptom. Post-mortem examination showed various changes, but none pathognomonic. The inoculation of a portion of the spinal cord into rabbits produced paralytic rabies, and examination of both human and rabbit material for Negri bodies proved positive. Knutti, in reviewing the literature, was unable to discover a similar case, that is, 'an ascending paralysis due to an acute destructive ascending myelitis caused by rabic virus', but pointed out that Remlinger had drawn attention to the fact that rabies is a possible cause of Landry's paralysis, and that in all fatal cases of acute ascending paralysis of unknown origin examination should be undertaken towards that end. Knutti was also of opinion that cases of the paralytic form of rabies have undoubtedly passed undetected through failure to perform a complete investigation. Roy (1936) in India gave an account of a child who was bitten by a dog, and 8 months later developed paresis of the legs, followed in 12 hours' time by paralysis and distention of the bladder, and on the following day by difficulty in swallowing and by hydrophobia. another day there was paralysis of the upper extremities, and death followed.

The diagnosis of paralytic rabies was clear, even in the absence of pathological examination.

These isolated instances of paralytic rabies, gathered from the comparatively scanty literature available locally, are few in contrast to the number of cases of classical rabies. The occurrence, therefore, in the island of Trinidad of a series of cases of the paralytic disease—the clinical details of which will be analysed elsewhere—should be of unique interest.

In 1919, the writer, while in charge of a rural district in the centre of the island, was called to inspect the body of a young female adult who had died without medical attendance. The history given by the relatives was so striking that, despite the fact that rabies was considered to be non-existent in the colony—though the last canine case had occurred in 1912—the brain of this girl was removed and forwarded to the then pathologist for examination for Negri bodies. The notes submitted at the time with the specimen were as follows:

'To the Medical Inspector of Health. I have forwarded to the Bacteriological Laboratory "ice-kept" specimens of the brain and cerebral fluid from a case upon which I performed a post-mortem examination yesterday at 6 p.m.

'The history of the case, which I obtained with great care from the mother, is as follows: about six (6) weeks ago, whilst washing some soiled linen at her mother's doorsteps, Bagwantiah, of Dyer Village, Brothers Settlement, age 14, was bitten on the middle of the left leg by a strange cat which sprang from beneath a cocoa house 10 feet distant. The cat also tried to bite a woman in the yard and was in their opinion "mad". A number of men with a great deal of difficulty—as the cat tried to bite them also—killed the animal with sticks and stones and buried it in the yard.

'On Wednesday last (7th) Bagwantiah complained of pain at the site of the wound—which had healed in about 3 weeks' time—and of pain in the whole left leg. On the night of the 10th the girl was compelled to take to her bed on account of the pain in the scar and leg, and she complained of feeling hot, though her mother says she had no fever. An hour or two later she could not walk owing to the "stiffness" of the leg and body (paralysis), and she then began to complain that her "throat was drying and stifling her" and that she was "very thirsty". Water was brought to her many times but she could never swallow. She continued asking for water—"I want plenty of water to drink"—in a husky voice, but she could not swallow. Without any other symptom of illness—except the great thirst, the inability to swallow and the paralysis—she died at 11 a.m., 11.5.19.

'A careful P.M. showed me nothing except congestion of the blood vessels of the brain and $\frac{1}{2}$ oz. serous fluid in the pericardial sac. Nothing was found in the throat; the spleen was slightly enlarged. A scar, 1 in. long and $\frac{1}{4}$ in. broad, on the leg was shown to me by her mother as the site of the bite.

'I was informed by some men who were in the room that one Mrs. Rosie Braxon was also bitten in the neighbouring village by a cat in March last, and she died with similar symptoms a month later. I shall make further inquiries.

'I shall be grateful for your opinion on the diagnosis, and for the result of your examination for Negri bodies and of the injection into rabbit of the brain specimen sent, if considered necessary.

'I regret I was not able to perform the P.M. earlier and to send specimens from spinal cord.

' J. L. PAWAN,

12.5.19.

'ag. District Medical Officer'.

Unfortunately, no report was received on the result of the examination for Negri bodies, but the history of a bite by an animal under such circumstances, and the manner of spread of the symptoms, led to a clinical diagnosis on the part of the writer of 'rabies with paralysis'. Hurst and Pawan (1931) described an outbreak of acute spreading myelitis in Trinidad due to the virus of rabies and transmitted by the vampire bat. From 1929 to the present date, 73 cases have been recorded. In every instance there was evidence of involvement of the spinal cord, with the development of paralysis. At periods varying from 3 weeks to 11 months following the bite by a vampire bat symptoms of paraesthesia, such as numbness, sensation of pins-and-needles, or pain, would develop, usually about the site bitten; then there would appear paresis, followed by a spreading paralysis, which would involve one limb, then another, then the trunk, and death would follow. In some cases the secondary stage of excitement so characteristic of classical 'furious' rabies was evident, but there was also present, as a prominent symptom, the spreading paralysis. Dysphagia was often absent, or appeared only as a terminal phenomenon. The paralysis was not of the polyneuritic, or disseminated, type, but was obviously dependent upon a transverse involvement, partial or complete, of the spinal cord. So pronounced may the paralysis be, with the absence of the classical features of rabies, that the diagnosis of myelitis due to a cause other than rabies was put forward in many instances, and it was only through histological and experimental examination that a correct diagnosis was possible. The paralysis may assume the form of a simple complete paraplegia with distension of the bladder, and may remain as such for several hours, without any other disturbing symptom or any evidence of classical rabies; then involvement of the other limbs and the respiratory muscles would follow, and death would intervene. There is no other record of such a series of cases of paralytic rabies in man as has been witnessed in Trinidad. The frequency with which the paralysis developed as the prominent clinical manifestation, and the obvious implication of the spinal cord in the form of a myelitis with involvement of a corresponding limb, render the adjectival qualification of 'paralytic' necessary in the designation of the disease. Gamaleia's contention, and Gowers' also, that there is a paralytic form of rabies differing in symptomatology from the classical disease, stands therefore unquestionable in face of the Trinidad outbreak. But there is also the possibility that isolated

cases of paralytic rabies are masquerading under the guise of Landry's or other forms of paralysis in the absence of a history of a bite or contact with a rabid animal. This should become a subject worthy of investigation both in countries where rabies is endemic and in those where the disease no longer occurs in its active 'furious' form in man or animal.

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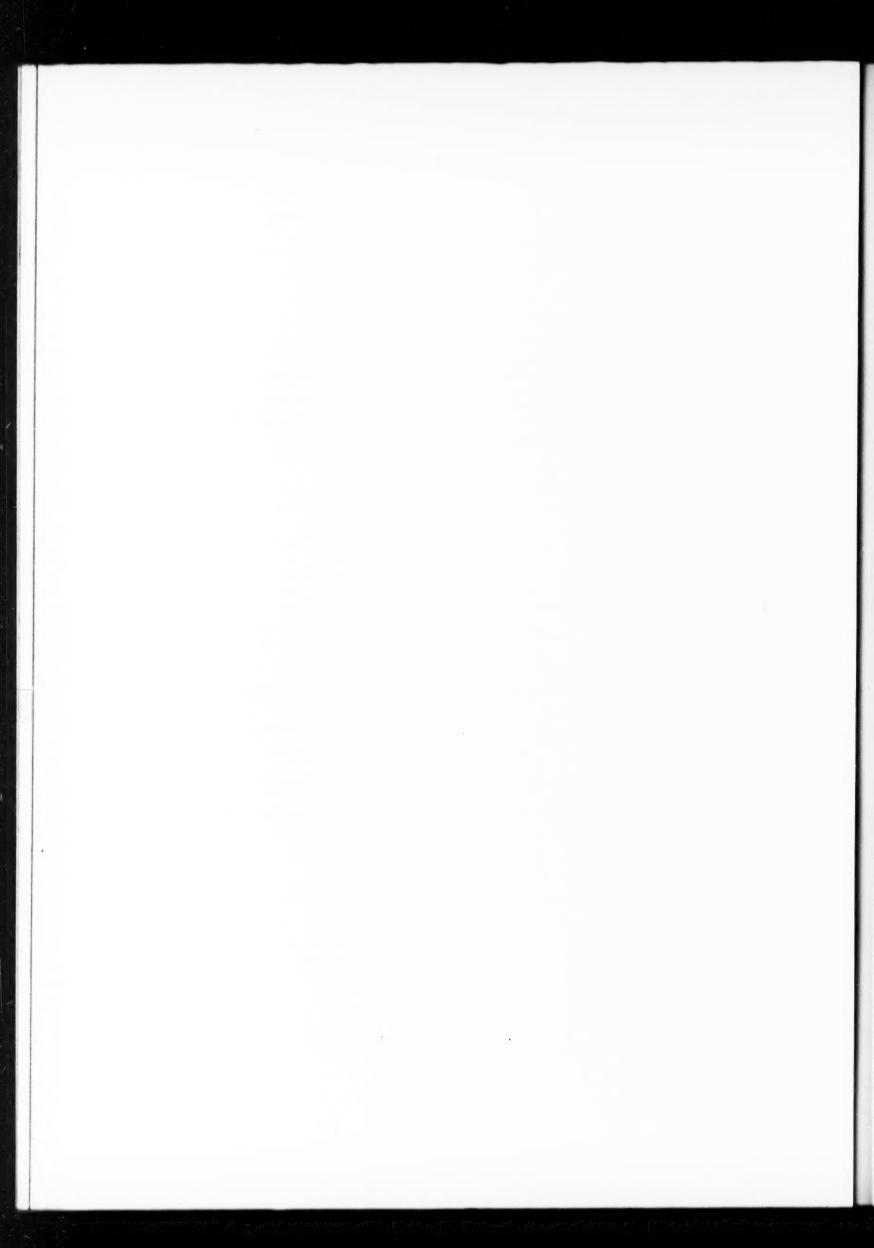
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A NOTE ON ANAPLASMA AND ON BARTONELLA STURMANI SP. NOV. IN THE BUFFALO

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On March 30th, 1938, three young buffaloes, 10–12 months old, were sent from the Huleh swamps to the laboratory of the Hebrew University, Jerusalem, by Dr. M. Sturman, veterinary surgeon of the Haklait Cattle Insurance Company. Blood-smears were examined daily until April 11th, and no abnormality was found. In order to determine the presence of any possible latent infection, the buffaloes were splenectomized on the following dates: buffalo no. 1, April 11th; buffalo no. 2, April 21st; buffalo no. 3, April 28th.

I. ANAPLASMA INFECTION

Observations on Buffalo No. 2

Before splenectomy: Red cells 6,120,000 per c.mm.

April 21st, 1938: Animal splenectomized.

April 22nd, 1938: Red cells 5,950,000 per c.mm.; Anaplasma morphologically indistinguishable from Anaplasma marginale found in the blood. Since the parasites appeared only one day after splenectomy, it is unlikely that the operation revealed a latent infection, as is frequently the case in calves in Palestine; and it is therefore probable that the operation was carried out during the incubation-period of an incipient infection.

Lestoquard (1932) recorded an experimental infection of buffaloes with *Anaplasma marginale*; the present note is the first record of a natural infection of *Anaplasma* in the same animal.

Parasites were found daily until May 19th, after which no trace of infection was revealed by examination of blood-smears. At the height of the infection, up to 70 per cent. of the red cells were infected, and from 1 to 3 parasites were found in each infected cell. The infection was accompanied by a progressive anaemia and a moderate leucocytosis, as shown in the following table.

Date	Red cells per c.mm.	Leucocytes per c.mm.	Percentage of infected red cells
3.5.38	5,300,000	31,200	33
6.5.38	3,890,000	19,600	60
9.5.38	3,800,000	20,000	65
10.5.38	2,500,000	24,000	70
13.5.38	2,200,000	24,000	48
15.5.38	2,000,000	17,200	37

The temperature during this period varied between 38.5° and 39.5° C. The animal showed no particular signs of distress, in spite of the severe anaemia.

Our findings in this naturally infected buffalo differ very considerably from those of Lestoquard in his experimental infections. Lestoquard found that in his experimental infections (one splenectomized and one normal animal) the Anaplasma tended to adopt a central position in the erythrocytes, and were in fact indistinguishable from Anaplasma centrale, although in the cow's blood used for producing these infections the parasites were marginal. He therefore considered the possibility of a sudden mutation of Anaplasma marginale into Anaplasma centrale by passage through a new host. In the case of our own buffalo (no. 2) we had the opportunity of studying innumerable parasites, nearly all of which were marginal and indistinguishable from Anaplasma marginale, only a small minority being situated at varying distances from the margin of the infected red cells.

Experiment No. 1

May 8th, 1938: Four normal calves on an experimental farm at Mikveh Israel were each inoculated with 5 c.cm. defibrinated blood taken four days previously from buffalo no. 2 (the blood was first tested by agglutination and found to be free from *Brucella abortus*). The calves were observed for five weeks, during which time no rise of temperature was noted.

May 30th, 1938: The blood of the four calves was examined and found negative. This experiment is inconclusive, because a latent *Anaplasma* infection in which no parasites are detected in blood-smears is common in calves in Palestine; these latent infections are revealed only by splenectomy, which the owner of these calves did not permit.

Experiment No. 2

May 4th, 1938: A splenectomized calf (no. 95) was inoculated with 5 c.cm. defibrinated blood taken from buffalo no. 2. This calf had been splenectomized on March 9th, and daily blood examinations up to the time of inoculation revealed no *Anaplasma* infection. On March 18th the calf was inoculated with *Theileria annulata* and *Babesia bigemina*.

May 13th, 1938: Anaplasma marginale was found in the blood of the calf and persisted until its death, which occurred on June 10th as a result of the Theileria and Babesia infections.

Experiment No. 3

May 4th, 1938: A normal non-splenectomized calf (no. 89) was inoculated with 5 c.cm. defibrinated blood from buffalo no. 2. Anaplasma marginale was

found in its blood on May 16th and persisted until May 23rd, when the parasites disappeared and did not recur.

Experiment No. 4

June 21st, 1938: Buffalo no. 2, after recovering from a *Bartonella* infection, which will be described below, and about one month after its blood was found negative for *Anaplasma*, was inoculated with 90 c.cm. blood taken from a calf during a severe *natural* infection of anaplasmosis. The buffalo was observed until July 22nd, when it died as the result of an accident, and no *Anaplasma* was found. It is believed that spontaneous recovery from its natural infection had rendered it immune.

Observations on Buffalo No. 3

Before splenectomy: Red cells 6,500,000 per c.mm.

April 28th, 1938: Animal splenectomized.

April 29th, 1938: Red cells 6,400,000 per c.mm.

July 14th, 1938: Anaplasma indistinguishable from Anaplasma marginale found in the blood. Since there was a considerable interval between splenectomy and the appearance of the parasites, it is possible that the animal was naturally infected by Boophilus annulatus subsequent to splenectomy. Parasites were found in the blood till July 22nd, when they disappeared. The course of the infection is shown in the following table.

Date	Red cells per c.mm.	Leucocytes per c.mm.	Temperature	Percentage of infected red cells
15.7.38	6,350,000	26,000	37·7° C.	38
17.7.38	6,150,000	22,000	37⋅8° C.	65
18.7.38	6,290,000	14,000	39⋅5° C.	72
19.7.38	5,390,000	15,000	39⋅5° C.	70
20.7.38	5,000,000	16,000	38·7° C.	68
21.7.38	4,520,000	18,000	38·8° C.	28

In this case too the parasites were mainly marginal and quite indistinguishable from *Anaplasma marginale*. Recovery from the anaemia was relatively rapid, and by August 4th the red-cell count had returned to normal.

II. BARTONELLA STURMANI SP. NOV, INFECTION

Observations on Buffalo No. 2

On May 22nd, 1938, Bartonella was found in blood-smears of buffalo no. 2. Morphologically this Bartonella was similar to Bartonella bovis Donatien and

Lestoquard, 1934 (syn. Bartonella sergenti Adler and Ellenbogen, 1934) and Bartonella muris. The course of the infection was as follows:

Date	Red cells per c.mm.	Leucocytes per c.mm.	Percentage of infected red cells
22,5,38			23
23, 5, 38	1,890,000	18,000	$92 \cdot 5$
24,5,38	1,750,000	19,000	30
25,5,38		• • •	19
26,5,38			7.5

On May 27th and subsequently no parasites were found in the blood.

The number of parasites per infected cell varied from 1 to 15, and were found individually, scattered irregularly in clumps, and in some cases in chains stretching across the cell. Parasites were also found at the periphery of the cell, and during the height of the infection, when more than 90 per cent. of the red cells were infected, some were also found free in the plasma. The organisms were usually bacillary in shape, but cocco-bacillary and almost coccoid forms were also seen; their length varied from 0.5μ to 1.5μ . Around some individual parasites a white halo was found, representing circumscribed areas of dehaemo-globinization.

No particular symptoms accompanied the infection, in spite of a temperature of from 39.5° C. to 40.5° C., and of the anaemia which was possibly the result of the previous infection with *Anaplasma marginale*. The bleeding-time was prolonged, and slight injuries (such as pricking the ear for making smears) were followed by copious and prolonged haemorrhage—a very unusual phenomenon in splenectomized animals, in which the blood generally coagulates more rapidly than in normal animals.

Experiment No. 5

May 25th, 1938: Two splenectomized rabbits were each inoculated with 5 c.cm. defibrinated blood from buffalo no. 2. The rabbits were examined daily until July 4th and were free from *Bartonella* during this period.

Experiment No. 6

May 26th, 1938: Ten c.cm. defibrinated blood from buffalo no. 2, taken on the previous day (19 per cent. of red cells infected) and kept on ice, were inoculated into a splenectomized calf (no. 95), which had been successfully infected with *Anaplasma marginale* from the same buffalo. Ten c.cm. defibrinated blood taken on May 23rd (92.5 per cent. of red cells infected) were inoculated into the same calf. The calf had been splenectomized on March 9th. Daily examinations revealed no *Bartonella* either before or after inoculation.

June 10th, 1938: The calf, as stated above, died as a result of an experimental infection of *Theileria annulata* and *Babesia bigemina*.

It should be noted that an infection of *Theileria annulata* in itself sometimes reveals a latent infection of *Bartonella* in calves, and that splenectomy without *Theileria annulata* is also sometimes sufficient for the same purpose. Nevertheless, in spite of the splenectomy and the infection of *Theileria annulata*, no *Bartonella* was found in this case.

Experiment No. 7

May 25th, 1938: Two splenectomized hamsters were each inoculated subcutaneously and intraperitoneally with 5 c.cm. blood taken from buffalo no. 2 on May 23rd and kept on ice. The blood of the animals was examined daily until July 4th, and no parasites were found.

Experiment No. 8

May 25th, 1938: Two splenectomized hamsters were each inoculated as above with 5 c.cm. blood taken on the same day from buffalo no. 2. The blood of the animals was examined daily until July 4th, and no parasites were found.

Experiment No. 9

May 31st, 1938: Numerous specimens of *Haematopinus tuberculatus* were removed from buffalo no. 2, emulsified and inoculated into buffalo no. 3. No *Bartonella* were found in buffalo no. 3 during an observation-period of 14 weeks.

Experiment No. 10

May 31st, 1938: An emulsion of *Haematopinus tuberculatus* from buffalo no. 2 was inoculated into a splenectomized normal calf (no. 101). Daily examination until September 1st revealed no *Bartonella*.

Experiment No. 11

June 21st, 1938 (a month after the blood became negative for *Bartonella* by microscopical examination): Three hundred c.cm. defibrinated blood of buffalo no. 2 were inoculated into buffalo no. 1.

OBSERVATIONS ON BUFFALO NO. 1

Before splenectomy: Red cells 7,750,000 per c.mm.

April 11th, 1938: Animal splenectomized.

April 12th, 1938: Red cells 7,700,000 per c.mm.

July 14th, 1938: Bartonella appeared in the blood, and 5 per cent. of the red cells were infected.

July 15th, 1938: Only 1 per cent. of the red cells infected.

July 16th, 1938: No parasites found.

There were from 1 to 15 parasites per infected cell. The infection was accompanied by distinct symptoms: the animal refused food, and the temperature rose to 40° C. on July 14th. There was a slight but distinct resultant anaemia: July 14th, red cells 7,780,000 per c.mm.; July 17th, 5,730,000 per c.mm. Two weeks later the red-cell count returned to normal. During the infection, the bleeding-time increased markedly, as in the previous case, and a slight trauma of the skin was followed by prolonged and copious haemorrhage.

Experiment No. 12

July 14th, 1938: Seventy-five c.cm. defibrinated blood from buffalo no. 1 were inoculated into a splenectomized calf (no. 99). This animal had been splenectomized on May 5th, and on May 9th was inoculated with Theileria annulata and Babesia bigemina. Daily examinations until September 12th revealed no Bartonella.

The above experiments show that the Bartonella of the buffalo is not transmissible by massive blood inoculation into calves, even under the most favourable circumstances for the development of an infection of Bartonella bovis (i.e., splenectomy and infection with *Theileria annulata*), but that it is transmissible from buffalo to buffalo by direct blood inoculation. The Bartonella of the buffalo is therefore distinct from Bartonella bovis and constitutes a new species, for which we propose the name Bartonella sturmani, in honour of our friend, Dr. M. Sturman.

SUMMARY AND CONCLUSIONS

1. Natural infection with Anaplasma in splenectomized buffaloes is described. The parasites, morphologically indistinguishable from Anaplasma marginale, were transmissible to calves by direct blood inoculation. Inoculated calves developed an infection indistinguishable from natural infections of Anaplasma marginale.

A Bartonella is described from a splenectomized buffalo. Bartonella can be transmitted by direct blood inoculation from buffalo to buffalo, but not from buffalo to calf. The name Bartonella sturmani is proposed for the species found in the buffalo.

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SOME SUCCESSFUL TRIALS OF PROSEPTASINE AS A TRUE CAUSAL PROPHYLACTIC AGAINST INFECTION WITH PLASMODIUM FALCIPARUM

BY

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While certain workers (de León, 1937; van der Wielen, 1937; Hill and Goodwin, 1937; Pakenham-Walsh and Rennie, 1938; Chopra and Das Gupta, 1938) have recently recorded that the sulphonamide compounds have some curative action in malarial infections in man and in monkeys, other reports have not been so encouraging (Hall, 1938; Faget et al., 1938; Kingsbury, 1938).

A few trials have been made of the value of proseptasine (May and Baker 125) at Horton Hospital, Epsom. The drug is said to be 'a benzyl derivative of sulphanilamide (para-benzylaminobenzenesulphonamide)', and one of 'the least toxic of all the sulphonamide compounds' (Cokkinis, 1938). Treatment with this drug was found to ameliorate the clinical manifestations of infection with the Roumanian strain of *P. falciparum* at present used in this hospital in the practice of malaria therapy. While proseptasine was observed to cut short acute attacks of this fever, the disease tended to relapse at a later date, in spite of large initial doses. The effects of this drug upon acute infections with the Madagascar strain of *P. vivax* were less encouraging.

On account of the action which this compound had shown upon infections with our strain of *P. falciparum*, it was decided to try whether it has any value as a true causal prophylactic† against mosquito-borne infections of this parasite.

^{*} This work was carried out at the London County Council Malaria-Therapy Centre, and at the Ministry of Health's Malaria Laboratory, Horton. The authors desire to thank the authorities of both these organizations for the facilities placed at their disposal. The senior author also wishes to acknowledge his indebtedness to the Royal Society of London and to the London School of Hygiene and Tropical Medicine, for the grants which have enabled him to continue his research work.

[†] We use the term 'true causal prophylaxis' to mean the complete prevention of the development of any schizogonic forms, as a result of the radical destruction of all the sporozoites, or of any intermediate stages between the former and the latter, i.e., the 'x' therapy of the Third General Report of the Malaria Commission of the League of Nations (1933). This term is used in contradistinction to that type of so-called 'prophylaxis' in which the inoculated sporozoites are able to develop up to the schizogonic or trophozoite stages (i.e., to produce infection), at which stages the 'prophylactic treatment' destroys the infection radically before it is capable of producing any clinical manifestations in the host. The latter is rather in the nature of 'early curative treatment' of the infection than a prophylaxis of it, in the true sense of the term, i.e., a prevention of infection.

The Fourth General Report of the Malaria Commission of the League of Nations (1937) on malaria treatment summarizes the work which had been done on true causal prophylaxis prior to that time, and more especially the work of James (1936). The conclusions reached by the Commission were as follow (p. 898): 'While there is some evidence to suggest that atebrin in large but harmless doses, and plasmoquine in doses of a toxic nature, may exert some degree of true causal prophylactic effect under certain conditions and with certain strains of P. falciparum, yet it is difficult or impossible with our present knowledge to rule out the possibility that this action may be upon the schizogenic forms of the parasite in red corpuscles, rather than upon the sporozoites or upon an intermediate stage between sporozoites and trophozoites.' Ciuca et al. (1937b, 1938) also produce evidence to show that the successful results reported with prophylactic courses of atebrin or quinine lasting more than about 8 days, were probably due to the action of these drugs upon the schizogonic forms which eventually develop from the sporozoites, rather than upon the latter forms or upon some hypothetical intermediate stages. Such an action would, therefore, be rather in the nature of 'early curative treatment' than 'true causal prophylaxis'.

It seems to us probable, however, that in the successful results reported by James (1936) with large doses of plasmoquine in *falciparum* infections, the action was a true causal prophylactic one, for this drug *per se* has not been found to have any destructive effect upon the asexual or schizogonic stages of *P. falci*-

parum.

The experiments carried out by Ciuca et al. (1937a) suggest that, in falciparum infections produced by sporozoites, the schizogonic forms of the parasite
cannot be detected in the peripheral blood before the 5th day after inoculation.
In the present work, therefore, the administration of the prophylactic drug was
never continued later than the day following that on which the infected insects
were allowed to bite the patient. In this way it was hoped to limit the action of
the drug to the sporozoites and the hypothetical forms intermediate between
these and trophozoites, thus excluding, as far as possible, any 'early treatment'
effects upon such schizogonic forms as would develop later if infection were not
prevented.

MATERIAL AND METHODS

The strain of *P. falciparum* employed was a Roumanian one kindly supplied by Professor Ciuca of the Cantacuzène Institute, Bucharest. The insects used were specimens of *Anopheles maculipennis* var. *atroparvus* infected with this parasite (Sinton, 1938a). In order that there should be no doubt that the patients received a potentially infective dosage of sporozoites, the mosquitoes used were heavily infected and a large number were applied. About 90 per cent. of the batch of insects used showed infection on dissection, and many showed 100 or more cocysts upon their mid-guts. The quantum of sporozoites

inoculated by each infected insect must, therefore, have been large, and the dosage of sporozoites given by 15–20 of such insects must have been much greater than that which would commonly be inoculated at one time in nature. The value of the drug as a true causal prophylactic was on this account put to a very severe test. We are indebted to Pharmaceutical Specialities (May and

Baker) Ltd., for the supply of proseptasine used.

The bloods of the patients were examined very carefully at least once daily by the thick-film method for 4 weeks, starting one week after the mosquitoes had bitten them, i.e., the earliest period at which, according to Ciuca et al. (1937a), one would expect to be able to detect falciparum parasites in the peripheral blood by microscopical examination. If no signs of infection were detected by the end of the 5th week, the examinations were reduced to at least twice weekly for another 3 weeks, making a total of 7 weeks of careful blood examination, after which the patients were examined at least once weekly, and always for several consecutive days if any signs of illness were reported.

The temperatures of the patients in tests I and II were taken 4-hourly for one month from the time of infection, while in test III they were taken twice daily for the first week and then 4-hourly. After a month, a temperature-record

was made twice daily for another 3 weeks, i.e., a total of 7 weeks in all.

RESULTS

The results are summarized in the following table (p. 40), and the details are as follow:

TEST I

Three non-immune patients (cases 1, 2 and 3) were given orally three doses of 2 gm. proseptasine at about 4-hourly intervals for one day. On the following day, half an hour after a further dose of 1.5 gm., these patients were bitten by 15, 20 and 15 infected mosquitoes respectively. Four hours later a further dose of 2.5 gm. was given, and 2.0 gm. after another 4 hours. This made a total of 7.5 gm. proseptasine administered during the 24 hours before the infecting bites, and 4.5 gm. during the next 8 hours (total amount 12 gm.).

Result. These patients have now been under observation for a period of 71 days, and, except for a single parasite observed in the blood of case 3 on the 12th day after the bites, no signs of infection, either clinical or parasitological, have been discovered. Another patient, as a control, was bitten on the next day by 20 mosquitoes from the same batch of insects, following which a severe attack of malaria developed after an incubation-period of 11 days.

TEST II

Two more patients (cases 4 and 5) received 3.0 gm. proseptasine thrice daily for one day. On the following morning, half an hour after another dose of 3.0 gm., they were bitten by 16 and 20 mosquitoes from the same batch as that used in test I. The same dosage of the drug was repeated 4 and 8 hours

later, and again thrice daily on the next day. These two patients had, therefore, a dosage of 12 gm. during the 24 hours before the infecting bites, and an additional 15 gm. during the next 32 hours (total amount 27 gm.).

Result. Case 4 developed fever and parasites on the 22nd day after the infecting bites, while case 5 has remained free from detectable infection up to the end of 65 days.

TEST III

Three other patients (cases 6, 7 and 8) were given three doses of 3.0 gm. proseptasine at about 4-hourly intervals during one day only. Following the last dose of the drug, case 6 was bitten by 30 infected mosquitoes after an interval

TABLE

	Dosage of drugs in gm. on days :		Inoculation				
Case no.	1	m. on days	3	No. of insects biting	Time after first dose of drug	Total amount of drug before inoculation	Result
1	6	1.5 † 4.5	0	15	24 hours	7·5 gm.	Nil (observation-period 71 days)*
2	6	1.5 † 4.5	0	20	24 ,,	7.5 ,,	
3	6	1.5 † 4.5	0	15	24 ,,	7.5 ,,	117 11
4	9	3.0 † 6.0	9	16	24 ,,	12.0	Attack after 22 days
5	9	3.0 † 6.0	9	20	24	12.0 ,,	Nil (observation-period 65 days)*
6	9†	0	0	30	7½	9.0 ,,	Attack after 15 days
7	9	0†	0	20	32 ,,	9.0 .,	Nil(observation-period 52 days)*
8	9	0	0†	20	56 .,	9.0 .,	Attack after 16 days

[†] indicates time of mosquito bites.

of half an hour, case 7 by 20 mosquitoes after 24 hours, and case 8 by 20 mosquitoes after 48 hours. A control case was bitten by 15 mosquitoes at the same time as case 8.

One insect from each of the lots fed on these patients was dissected immediately after biting, and was found to have numerous sporozoites in the salivary glands.

Result. The control patient developed an acute attack of malaria after an incubation-period of 13 days. Cases 6 and 8, who were bitten half an hour

^{*} on November 26th, 1938.

and 48 hours after the last dose of the drug, showed severe infections after incubation-periods of 15 and 16 days respectively. Case 7 has, however, shown no signs of infection up to the present (observation-period 52 days), although carrying on her usual work and not confined to bed.

TOXICITY OF PROSEPTASINE

At a meeting of the Medical Society of London, Willcox (1938), in opening a discussion on the use of the sulphonamide compounds, stated that 'Toxic symptoms of a mild type . . . were extremely common when full doses of these drugs were being taken; they occurred in 50 per cent. or more of cases. . . . Toxic symptoms of such gravity as to cause death were infrequent, but idiosyncrasy had always to be remembered and extreme watchfulness and care should be exercised'. He pointed out, moreover, that 'drugs of the sulphanilamide group should be taken only under medical supervision'.

As any malarial prophylactic treatment which necessitates the confinement of the patient to bed would be of little practical value in most circumstances, the majority of our cases were allowed up. In only two of these were any by-effects of the drug observed: these were cases 6 and 7, who had each received

three 4-hourly doses of 3 gm. of the drug during one day.

Case 6 developed nausea and vomited a few hours after the last dose of the drug. The nausea and headache continued into the next day, but quickly disappeared. Case 7 complained of nausea and headache a few hours after the treatment was finished, and, on the following morning, showed an erythematous rash on the front of the shins. This faded after 24 hours.

The ingestion of sulphur is said to increase the toxicity of the sulphanilamide compounds, and special dietetic measures are recommended to reduce the danger from this source. Apart from any other considerations, such necessary dietetic precautions alone would exclude these drugs as being of practical value as causal prophylactics in the field, except under very specialized conditions of limited duration.

DISCUSSION

Of the eight patients given causal prophylactic treatment with proseptasine, five have not yet developed malaria, although they have been under careful clinical and parasitological observation during periods of from 52 to 71 days.* The facts that the control cases bitten by the same batch of infected insects developed the infection within the limits of the normal incubation-period, and that with such a heavy dosage of *falciparum* our routine results have previously shown no such failures to infect, appear to rule out all other factors, except the prophylactic action of the drug, as the cause of the successful results obtained in our tests.

^{*}These periods were 126, 139 and 145 days respectively on February 8th, 1939.

Five out of the six cases who were bitten 24-32 hours after the first dose of the drug did not develop the disease, while the two cases bitten after $7\frac{1}{2}$ and 56 hours respectively did so, even although larger amounts of the drug had been given than in some of the former cases. This suggests that the results may possibly be influenced by the rates of absorption and excretion of the drug. It is possible that in case 6 the concentration of the sulphanilamide element in the blood had not reached a lethal level during the period when the sporozoites or their immediate progeny were in a vulnerable condition or position, while in case 8 the rapid excretion of the drug may have reduced the drug-concentration below such a level.

In case 4, the only 24-hour case which developed the disease, the incubation-period was longer than normal (22 days). Ciuca *et al.* (1938) also record prolonged incubation-periods in *falciparum* infections which had received prophylactic treatment with atebrin. They attribute this condition to some 'debilitating' action of the drug upon the sporozoites, or upon the hypothetical intermediate stage between these and trophozoites. Such an explanation might be applicable to case 4.

Under the conditions of our tests, proseptasine appears to have a true causal prophylactic action against the Roumanian strain of *P. falciparum* used by us. That such an action, if present, is not merely due to 'early curative treatment' is supported by the facts that (1) in three of the successful cases treatment was not continued for more than 8 hours after the infective mosquito bites, while in another case it had been stopped 24 hours previously (the well-known rapid excretion of the sulphonamide compounds suggests that the blood-concentration of the drug could not have continued long enough in these cases to affect the schizogonic forms which develop later if infection occurs); and that (2) the numerous relapses seen in our clinical tests of the therapeutic value of this drug suggest that even greater and more prolonged dosage with proseptasine will not often give rise to a complete radical destruction of the schizogonic forms of this strain of parasite.

It is of interest to note that, although in case 3 a parasite was detected in the thick film on the 12th day, yet the patient did not develop the disease, while in case 8, although parasites were seen on the 8th day, yet they could not be found again until the 16th day, when the febrile attack commenced. The latter finding is unusual in our experience, in which the detection of parasites in primary infections of malignant tertian malaria is seldom separated by more than one or two days from the onset of the febrile attack. Can any tentative suggestions be made to account for these findings?

An editorial in the *British Medical Journal* (1938) reviews the work on the mechanism of the action of the sulphanilamide compounds. The effects of these drugs in bacterial infections are believed not to be directly bacteriocidal but to be mainly bacteriostatic. When the rate of multiplication of the microorganisms has been stopped or held in check, it is considered that the natural

defensive mechanism of the host deals with the bacteria. If this be sufficient to destroy the parasites completely, or to reduce them to numbers below symptom level, a cure results, either permanent or temporary. Some workers consider that the macrophage system may take a predominant part in this destruction, an hypothesis which is interesting in view of the part which the macrophages play in the cure of malarial infections (Sinton, 1938b).

We have no information whether sulphanilamide causes a similar retardation of parasitic development in malarial infections. If it does, the results observed in case 3 might be explicable on the assumption that all the precursors of the asexual cycle of the parasite were not killed and that some of them reached the schizogonic stage, but were so 'debilitated' that the natural bodily defences destroyed them before they could multiply to symptom level. On the other hand, in case 8 this destructive action was not completely successful, and, although the parasite increase was retarded, they eventually multiplied up to such numbers that clinical manifestations were produced.

Our results appear to indicate that proseptasine has some true causal prophylactic effect against the Roumanian strain of *P. falciparum* used in our tests. These results require confirmation, and further work is in progress.

Even if proseptasine has a true causal prophylactic action, this would not, however, appear to have much practical value, (1) because its protective action appears to be of such very short duration after the cessation of treatment, while its toxic properties contra-indicate more continuous administration, (2) because of the need for medical supervision of its use, and (3) because of the dietetic limitations which should be enforced during any course of treatment.

The results are, however, of considerable scientific interest, if confirmed, as showing that drugs of this type have apparently a definite action upon the pre-schizogonic stages of at least one strain of the malignant tertian malaria parasite. Except possibly for plasmoquine in toxic doses, no other drug is known which has this action. This suggests fresh lines for chemotherapeutic research. It is possible that some other allied compound of the sulphonamide group may be discovered, which, while being therapeutically effective as a true causal prophylactic in malaria, may have a lowered toxicity and a more prolonged action.

CONCLUSIONS

Under the conditions of our trials, proseptasine, given about 24 hours before a patient is bitten by mosquitoes heavily infected with a Roumanian strain of *P. falciparum*, appears to have some true causal prophylactic action. These findings require confirmation.

On account of the precautions necessary during treatment with the sulphonamide compounds, and the apparently short duration of the prophylactic action observed, these results do not appear capable of practical application at present.

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THE BEHAVIOUR OF LEISHMANIA CHAGASI IN PHLEBOTOMUS PAPATASII

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In a previous paper (Adler and Theodor, 1927) a method was described of infecting *Phlebotomus papatasii* with various species of *Leishmania* by feeding the insects through a membrane on suspensions of flagellates. Unfortunately, up to the present this method has proved applicable only to *P. papatasii*; *P. sergenti* and sandflies of the *major* group very rarely feed through artificial membranes. In the case of *P. papatasii*, relatively few sandflies feed through the membrane unless the fluid on which they are allowed to feed contains red cells, but a large, though varying, proportion of sandflies (up to 80 per cent.) feed if the membrane is first covered with a solution of cane sugar, which is allowed to dry on its surface. Apparently tasting the sugar stimulates the acts of piercing and of pumping fluid into the alimentary canal.

The advantage of the above method of infecting sandflies is that the infectivity of various strains and species of *Leishmania* can be compared quantitatively, since suspensions of flagellates can be made to any desired concentration and

the volume of fluid ingested by a sandfly is fairly constant.

Some strains of L. tropica transmitted in nature by P. sergenti (e.g., the Cretan strain; see Adler, Theodor and Witenberg, 1938), as well as both L. donovani and L. infantum (see Adler and Theodor, 1931), produce a negligible infection-rate in P. papatasii when fed on man or on naturally infected dogs. The sandfly is naturally resistant to these strains, but the resistance can be reduced either by feeding them through membrane on large numbers of flagellates, or, in the case of the Cretan strain of L. tropica, by reducing the proportion of serum in the nutrient suspension (Adler, Theodor and Witenberg, 1938). Once the resistance of the insect has been broken down, the behaviour of the flagellates in their artificial host—P. papatasii in the case of all Old World species of Leishmania—gives a fair indication of their behaviour in their natural host, except that short-form infections, such as occur in P. perniciosus infected with L. infantum (i.e., thin flagellates 4–10 μ long, with a flagellum as long as or longer than the body), have not yet been found in P. papatasii infected with this species.

Owing to the kindness of Dr. A. Marques da Cunha, of the Oswaldo Cruz Institute of Brazil, we received a number of strains of the recently discovered L. chagasi, the causative agent of visceral leishmaniasis in Brazil.

Cultures have been maintained on Locke serum agar and on Shortt's medium, and on these media they behaved like other species of *Leishmania* of human and canine origin.

One strain (Isaias), isolated in September, 1937, was used for feeding experiments with laboratory-bred *P. papatasii*. Suspensions of flagellates of various concentrations from 300 to 6,000 per 0·1 c.mm. were used. The infection-rate in the sandflies depended, as was to be expected, on the concentration of flagellates used; out of 54 sandflies fed on suspensions of 300 flagellates per 0·1 c.mm., only 13 became infected (24 per cent.), while, out of 189 sandflies fed on suspensions of 1,000–2,000 flagellates per 0·1 c.mm., 134 became infected (71 per cent.), and in still higher concentrations 185 out of 209 sandflies became infected (89 per cent.).

In all the experiments the sandflies ingested far more flagellates than they could obtain under natural conditions by feeding on human or canine cases; and the fact that suspensions of 300 flagellates per 0·1 c.mm. produced an infection-rate of only 24 per cent. shows that *P. papatasii* could not be an efficient vector of *L. chagasi* in nature. This is in marked contrast to the findings in Jericho strains of *L. tropica*, which produce an infection-rate of 80 per cent. in *P. papatasii* sandflies fed on emulsion of 100 flagellates per 0·1 c.mm., and 90 per cent. to almost 100 per cent. in sandflies fed on suspensions of 300–400 flagellates per 0·1 c.mm. (Adler, 1932).

L. chagasi resembles both L. donovani and the strains of L. infantum which we isolated in Italy, since these strains also produced a relatively low infection-rate in P. papatasii fed on emulsions of 300–400 flagellates per 0·1 c.mm. (Adler and Theodor, 1931).

Once established in *P. papatasii*, the behaviour of *L. chagasi* is as follows. The flagellates multiply in the midgut, and many of them ascend the anterior part of the cardia, which may be choked with parasites. Flagellates pass through the oesophageal valve into the oesophagus as early as three days after the infecting feed. The tendency towards an anterior position is pronounced, so much so that, in slight infections, the flagellates may be confined entirely to the upper part of the cardia while the stomach is free from flagellates, or the cardia may be choked with parasites while the stomach is relatively free. As in the case of the human and canine leishmaniasis of the Old World, *L. chagasi* attaches itself by the extremity of the flagellum to the epithelium of the cardia. The flagellates in general avoid the hindgut, except in the case of very heavy infections.

In sections of whole infected sandflies, the flagellates were traced as far as the anterior end of the pharynx after 5 days at 27° C. At lower temperatures (19°-21° C.), in 14 out of 50 infected sandflies the flagellates were confined to the stomach 4-8 days after the infecting feed. A similar finding has also been noted in the case of Maltese strains of *L. infantum* and in *P. perniciosus*, in which as many as 25 per cent. of the infections may be confined to the stomach; but in the case of *P. perniciosus* the distribution is independent of temperature, since

it occurs only at the beginning and middle of the sandfly season, even at a temperature of 29° C., and is rare at the lower temperature prevailing in October and November (Adler and Theodor, 1935). This, so far, is the only difference which we have noted between the behaviour of L. chagasi and that of L. infantum in P. papastasii; but, since this observation is for the present confined only to a single strain of L. chagasi, no final conclusions can be drawn. The similarity of behaviour of L. infantum and L. donovani in P. papatasii to that of L. chagasi leaves no doubt that the vector of the latter species will also prove to be a sandfly.

SUMMARY

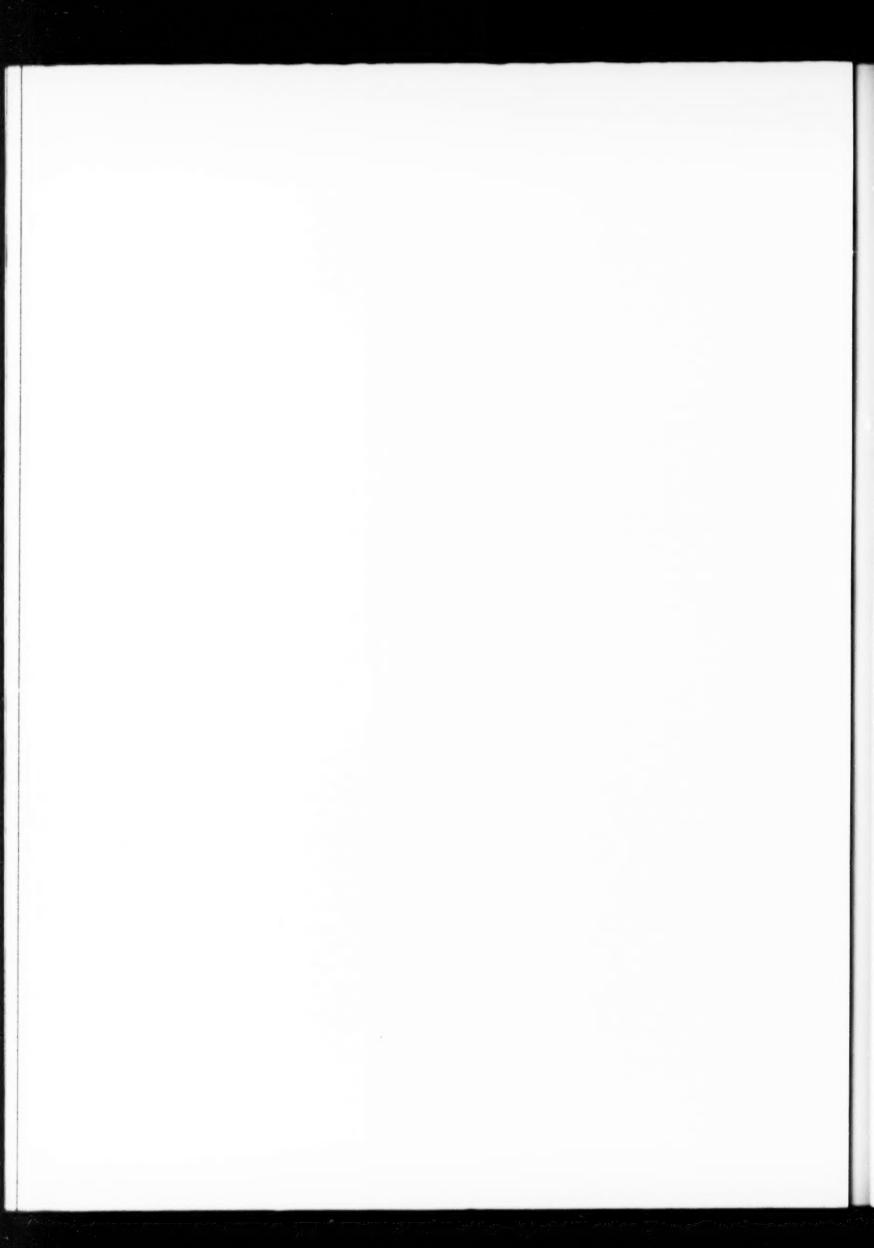
Phlebotomus papatasii has been infected with L. chagasi by feeding on suspensions of flagellates.

An infection-rate of 24 per cent. was produced in sandflies fed on 300 flagellates per 0·1 c.mm., 71 per cent. in sandflies fed on emulsions of 1,000–2,000 flagellates per 0·1 c.mm., and 89 per cent. in higher concentrations.

The flagellates adopt an anterior position, and, attaching themselves to the epithelium of the cardia, pass through the oesophagus; they have been traced to the anterior end of the pharynx after 5 days.

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REPORT ON AN EXAMINATION OF THE SPLEEN- AND PARASITE-RATES IN SCHOOL-CHILDREN IN FREETOWN, SIERRA LEONE

BY

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(Received for publication January 27th, 1939)

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I. INTRODUCTION

Since 1915, examinations of blood, or of blood and spleen, of school-children in Freetown, Sierra Leone, have from time to time been carried out and the results put on record. Gordon and Davey (1932) give a summary of the findings from 1915 to the time of their own investigation in 1931. In 1930 McDowall did spleen and blood examinations, using one thin film for the parasite-rate. Her figures relate only to children up to the age of 8 years and those of 12 years. In 1933, the Medical Officer of Health examined 254 children at three schools, and found that 56 (22 per cent.) had malaria parasites in the blood. No details are given regarding age, except that the average age for males was 7.2 years and for females 6.7 years. No information is recorded about spleen-rate or type of parasite.

The present paper deals with a spleen and blood examination done in November and December, 1935, on school-children in Freetown, from the age of 3 to 10 years, the object of which was to ascertain the splenic index, and, in particular, to see if there had been any decrease which might be due to the drainage scheme. Reference to this scheme will be made later. The record follows the lines of Macdonald's paper (1926), whose investigation seems to be the most thorough of its kind ever done in Freetown. On account of the short

time allowed for the work, this investigation does not profess to have either the detail or the value of his work, and it is recorded as much for its future value as a comparison with other examinations as for any present worth. It does, however, give some useful and interesting information, especially regarding the percentage of the various species of *Plasmodium* seen in the blood, and raises important questions concerning possible results of drainage operations.

The examinations were done at the early part of the dry season, between November 20th and December 3rd, the clinical work being done by one of us (H.P.) and the blood examinations by the other. Nine schools in different parts of the town were visited, and the spleen of all children between the ages of 3 and 10 years were carefully examined and a thick blood-film taken. The name, address, age and sex of each child were noted, and the blood-films were numbered serially, to be examined later at leisure. No examination of temperatures was performed.

II. EXAMINATION OF THE SPLEEN

- (a) METHOD OF EXAMINATION. With the child facing the examiner, arms at sides, balanced equally on both feet and looking straight in front, mouth open and breathing quietly, the apex of the spleen was carefully located and marked with a grease pencil. The following measurements were then taken (in centimetres):
 - (a) Distance from apex to umbilicus.
 - (b) Distance from apex to mid-line.
 - (c) Distance from apex to left nipple.

These were corrected for the size of the child, using Macdonald's table. A note was made of the spleens 'palpable but not reaching the costal margin,' at costal margin,' and 'below umbilicus,' the two former being given the same values as in Macdonald's paper, namely, 15 cm. and 13 cm. respectively.

TABLE I

	T, 5- 25-10-21 \$1.000				Male	Female	Total
No. examined		• • •	 4 + 0	• • •	454	497	951
No. with palpable	spleen		 		202	210	412
Spleen-rate			 		44	42	43

(b) RESULTS OF EXAMINATION. Nine hundred and seventy-three children were seen, 20 being Syrians, 2 half-European, and the remaining 951 African. Except for a short note at the end, only the last group is referred to in this paper. Of these 951 African children, 412 had palpable spleens, so that the spleen index of Ross (1910) is 43 (Macdonald 56).

Table I shows the number, sex and spleen-rates. Only two had spleens reaching below the umbilicus.

In Table II are shown the schools at which the children were examined, the number from each school, and the percentage with palpable spleens.

TABLE II

Scho	ol		No. examined	Percentage with palpable spleen		
Buxton		 	156	3.2		
Christ Church		 	87	38		
Samaria		 	29	38		
Bathurst Street		 	110	44		
Cathedral		 	158	42		
St. Joseph's		 	166	55		
Ebenezer		 ***	29	59		
Tabernacle		 	144	42		
Model		 	72	49		

The first two schools on the list are in the West Ward and the remainder in the Central Ward.*

Table III shows the age-grouping, taking two-yearly periods.

TABLE III

Age	No. examined	Percentage with palpable splee
3-4	55	37
5-6	289	42
7-8	329	44
9-10	278	45
The same of the sa	951	43

Macdonald's investigation was made between July, 1925, and March, 1926—a period of eight months. He noted a hyperendemic and an endemic area, and points out that the latter was examined during the wet season and the early part of the dry season—normally the most malarious season of the year—and the former during the latter part of the dry season, when it is relatively healthy. As already stated, the present investigation was made at the end of November and beginning of December, i.e., in the most malarious part of the year.

^{*}This local information is given only for purposes of comparison, because of similar lists in Macdonald's paper and other reports.

Following his analysis, the cases were divided into two groups, those living in the extreme west (hyperendemic area) and those living in the central and eastern portion (endemic area).

(1) Endemic Area. Of the total 951 African children examined, 465 came from the endemic area, and of these 205 (44·1 per cent.) had enlarged spleens. (Macdonald's figure for children of 10 and under is 49 per cent.) The average distance of the spleen from the umbilicus, including palpable and costal margin spleens, was 10·4 cm. (Macdonald, 10·5 cm.).

Relation between the spleen and parasite findings. Of the 260 children without enlarged spleens, 78 (30.0 per cent.) had parasites in their blood, while of the 205 with enlarged spleens, 80 (39.0 per cent.) had positive bloods

(Macdonald, 27 and 56 per cent. respectively).

(2) Hyperendemic Area. Of the 486 cases in the hyperendemic area, 207 (42.6 per cent.) had palpable spleens, so that the spleen index of Ross is 43. Macdonald's figure is 71 per cent. The average distance of the spleen from the umbilicus was 10.0 cm. (Macdonald, 8.1 cm.)—approximately the same as in the endemic area.

Relation between the spleen and parasite findings. Of the 279 children without enlarged spleens, 82 (29.4 per cent.) had parasites in their blood, while of the 207 with enlarged spleens, 105 (50.7 per cent.) had positive bloods (Macdonald's figures are 66 and 75 per cent respectively).

III. EXAMINATION OF THE PERIPHERAL BLOOD

A single thick film was examined after staining with Giemsa, and 345 (36·3 per cent.) of the 951 showed malaria parasites (Macdonald, 51 per cent.). Below is the analysis of the parasite findings:

P. malariae						167 = 17.6 per cent.
P. falciparum						133 = 14.0 per cent.
Mixed infections	with P .	malariae	and I	. falcij	barum	40 = 4.2 per cent.
P. vivax						4 = 0.4 per cent.
Mixed infections						1 = 0.1 per cent.

One mixed infection diagnosed as *P. malariae* and ? *P. vivax* is included under *P. malariae*. No *P. ovale* were seen.

Macdonald used a single *thin* film. This difference in the two examinations is of great importance and will be referred to later.

Arranging these findings according to the number infected with each species of parasite, and expressing these figures as percentages of the total positives, we find the following:

P. malariae	 	 	 	208	(60·3 per cent.).
P. falciparum	 	 	 	173	(50·1 per cent.).
P. vivax	 	 	 	5	(1.4 per cent.).

Gordon and Davey (1932) have reported the change in the proportions of the various species of malaria parasite since Macdonald's investigation in 1925-26; the figures for *P. malariae* and *P. falciparum* above are almost the same as theirs, and show that the increase in *P. malariae* infections has been maintained. Gordon and Davey (1933) have also reported the same increase in quartan infections in the hyperendemic area in 1932.

Table IV shows the number and percentage of children infected at each agegroup, from 3 to 10 years.

TABLE IV

Age	No. examined	No. with positive blood	
3-4	55	21	38.2
5-6	289	112	38.8
7-8	329	119	36.2
9-10	278	93	$33 \cdot 5$
	951	345	36:3

(a) ENDEMIC AREA. Of the 465 children examined in the endemic area, 158 (34.0 per cent.) had infected bloods (Macdonald, 41 per cent.). Table V shows the analysis of the parasite findings for this area, the numbers infected with each parasite, and the percentages of the total positives.

TABLE V

	Spec	cies			No. positive	Percentage positive	No. infected in each species	Percentage of total positives
P. malariae					79	17.0	96	60.8
P. falciparum					62	13.3	78	49.4
P. vivax					-	-	1	0.6
Mixed P. mala	riae an	d P. fa	lciparui	n	16	3.4		-
Mixed P. mala	riae an	d P. vi	rax		1	0.2	_	

Age incidence of malaria findings. Table VI shows the percentage of children infected at each age-group from 3 to 10 years.

(b) HYPERENDEMIC AREA. Of the 486 children whose blood was examined, 187 (38.5 per cent.) had malaria parasites in their blood (Macdonald, 72 per cent.).

Table VII shows the analysis of the parasite findings for the hyperendemic area, the numbers infected with each parasite, and the percentages of the total positives.

TABLE VI

Age	No. examined		Percentage with positive blood
3-4	29	*)	41.4
5-6	143	51	$35 \cdot 7$
7-8	153	52	34.0
9-10	140	43	30.7
	465	158	34.0

TABLE VII

	Spec	cies			No. positive	Percentage positive	No. infected in each species	Percentage of total positives
P. malariae					88	18-1	112	59.9
P. falciparum					71	14.6	95	$50 \cdot 8$
P. vivax					4	0.8	4	2.1
Mixed P. mala	riae an	id P. fa	lciparu	m	24	4.9		

TABLE VIII

Age			Percentage with positive blood
3-4	26	9	34.6
5-6	146	61	41.8
7-8	176	67	38.1
9-10	138	50	$36 \cdot 2$
	486	187	38.5

Age incidence of malaria findings. Table VIII shows the percentage of children infected at each age-group from 3 to 10 years.

IV. CONCENTRATION OF MALARIA ROUND ANOPHELINE BREEDING-PLACES

The same two areas mentioned by Macdonald were studied; in area A 74 children were resident, 30 of whom had malaria parasites in their blood (41 per cent.); of 186 children in area B, 63 were positive (34 per cent.) (Macdonald's figures were 26 per cent. and 63 per cent. respectively). The spleen indices were 48.6 for area A, and 37.1 for area B.

V. TRUE INFECTION-RATE

Accepting the formula that the true infection-rate is equal to $p = \frac{100}{x}$ (where p is the parasite-rate per cent. and x the parasite-rate amongst those with enlarged spleens), it is interesting to compare our figures with those of Macdonald:

	Macdonald, 1926	1935
Whole series .	80.4	80.8
Endemic area .	73.2	87.2
Hyperendemic are	ea 96.0	75.9

VI. MALARIA IN SYRIAN CHILDREN

As has already been mentioned, 20 Syrian children were seen during the present investigation. Their ages were from 4 to 10 years, and 19 came from the endemic area. Eleven of the 19 had enlarged spleens, 2 being below the umbilicus; and 4 had positive bloods. The average distance of the spleen from the umbilicus was 8.7 cm. for the 9 whose spleens did not come below the umbilicus, compared with 10.4 cm. for the African children.

VII. PERMANENT DRAINAGE OPERATIONS IN FREETOWN SINCE 1930

Gordon et al. (1932) have given the history of the anti-mosquito measures in Freetown from their inception in 1899 up to the time of their investigation in 1930-31, and they conclude that these measures had resulted in a remarkable dimunition in the number of mosquitoes in Freetown during the previous 30 years. They quote Stephens and Christophers' (1900) statement, that the only source of the adult anophelines found was Sander's Brook; and they mention the commencement of the permanent canalization of this brook in 1930.

CANALIZATION OF SANDER'S BROOK. The scheme proposed was to canalize the brook by means of a concrete channel from the municipal boundary to its outfall in Kroo Bay, and to provide permanent surface drainage for the whole

area draining into this brook. The district to be dealt with comprised some 250 acres within the municipal area, and 290 acres outside it (Annual Medical and Sanitary Report for 1929).

Work on the canalization of Sander's Brook was commenced on February 12th, 1930, from its outfall at Kroo Bay, and the first section of the canal, i.e., from Kroo Bay to Joaque Bridge, was completed during that year, as well as two-thirds of the length of the second section from Joaque Bridge to Robert Street. Permanent drainage of several streets was also completed. In 1931 the second section of the canal and part of the third section from Robert Street to a point a little above West Street was completed, as well as several permanent street drains. During 1932, the 1931 programme was completed, and this brought the canal up to a point near Dundas Street.

Work on the canal was closed down early in the year 1932, owing to the suspension of the scheme for two years, and was resumed in 1934 upon an amended programme, which allowed of the scheme being spread over eight years. The main canal was extended from near Dundas Street, where work was suspended in 1932, to a point north-east of the Public Works Department yard; and, again, permanent surface-water drains were laid in several streets. The main canal of Sander's Brook was completed in 1935. The canal was extended to a point near the stone-crushing plant, where it divided into two smaller channels, one turning south for a short distance and the other running eastward for about 200 yards, where it terminated at a catchment basin at the foot of the hill where the stream enters the valley. Several streets were also permanently drained.

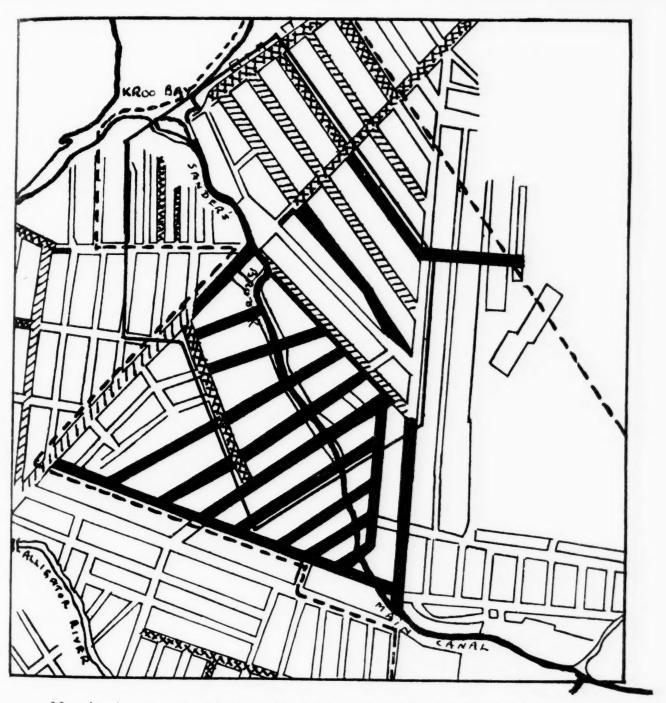
The scheme for permanent surface drainage of the streets in the Sander's Brook area is being continued.

VIII. DISCUSSION

The results of this investigation seem to show that there has been a marked decrease in the incidence of malaria in the hyperendemic area of Freetown, as shown by the spleen- and parasite-rates and the true infection-rate.

In comparing our figures with those of 1926, two things should be borne in mind. Firstly, Macdonald's figures related almost entirely to the ages 3 to 12 years (only 21 of his 1,059 children being above 13 years old); Gordon and Davey (1932) show that both Macdonald's hyperendemic area figures and their own indicate that from the age of 10 and 12 years respectively and onwards the level of the malaria incidence drops. And, secondly, Macdonald used a single thin film, whereas we used a single thick film. Both these factors would tend to make our figures higher than Macdonald's.

Again, the true infection-rate for the whole series in 1926 and 1935 is the same, but it is probable that ours is the truer figure and that Macdonald's figure would have been appreciably higher had he used thick films. The thick film also may account for the increase in the true infection-rate for the endemic area and the increase in the parasite-rate in area A.



Map showing area selected for canalization, area B, and Sander's Brook main canal.

Area B.

Permanent drainage, 1930-35.

Tar surfacing, 1930-35.

Work prior to 1930,

In the hyperendemic area the average distance of the apex of the spleen from the umbilicus is now the same as in the endemic area, and not, as in 1925-26, considerably less. The spleen-rate and average distance of the spleen from the umbilicus for the endemic area remain substantially the same as they were.

Of the children without enlarged spleen, 29.7 per cent. had parasites in their blood. Whereas in 1925-26 there were twice as many children with parasites in their blood in the hyperendemic area as in the endemic area, the figure in 1935 was the same for both areas.

During the time which has elapsed since Macdonald did his thorough survey until the time of our own investigation, much has been done to canalize permanently not only the main canal of Sander's Brook, but also the streets draining into this brook. Gordon and Davey (1932) reported that those portions of the anti-malaria drainage scheme which had been completed prior to its suspension had proved successful, as shown by a reduction in the number of anophelines captured in the streets drained. Since their breeding-places have been to a great extent removed by the drainage scheme, it is only to be expected that any concentration of malaria round those sites would also be reduced.

The widening of roads resulting from this scheme has had the effect of bringing more light and air into the area, thus tending to reduce hiding-places of mosquitoes.

It seems certain, therefore, that these drainage operations have been responsible for the reduction, and that the western part of Freetown should no longer be termed hyperendemic, but that the whole of the town should be regarded as an endemic area. This scheme will be completed in 1942, and we suggest that another thorough investigation then be done, and the 1925-26 and 1935 figures be compared; a study similar to that of Gordon et. al (1932) would be of value.

IX. SUMMARY

1. Nine hundred and fifty-one African children in nine Freetown schools were examined in November and December, 1935, records being kept of the presence and size of enlarged spleens, and of the presence of malaria parasites in the peripheral blood. The children were aged from 3 to 10 years.

Of these children, roughly half came from the western part of the town (hyperendemic area) and half from the central and eastern portion (endemic area). This was not done designedly, as the addresses were not classified until long after the examinations were completed.

2. The spleen-rate was found to be 43 for the whole series, $44\cdot1$ for the endemic area, and $42\cdot6$ for the hyperendemic area. The corresponding parasite-rates were $36\cdot3$, $34\cdot0$, and $38\cdot5$; and the true infection-rates $80\cdot8$, $87\cdot2$, and $75\cdot9$ respectively.

3. The children living in the area B, mentioned by Macdonald, were found to have a spleen-rate of 37·1 per cent. and a parasite-rate of 34 per cent.

- 4. The age incidence of the spleen-rates and parasite findings are given.
- 5. The increase of P. malariae infections at the expense of P. falciparum, reported by Gordon and Davey (1932), was found to be still present. P. vivax infections were present in very small numbers. No P. ovale were seen.
- 6. A brief account is given of the completion of the permanent canalization of the Sander's Brook main canal; reference is made to the scheme for the permanent drainage of the streets draining into Sander's Brook. This scheme is being carried on.
- 7. The conclusion is drawn that the hyperendemic area of Freetown no longer exists as such, but that all Freetown should now be regarded as an endemic area.

ACKNOWLEDGEMENTS.—We have to thank the Honourable the Director of Medical Services (Dr. W. P. H. Lightbody) for permission to publish this paper, and we acknowledge with gratitude the helpful advice and practical assistance given by Professors Gordon and Davey. We also express our thanks to Mr. A. R. Smee (Provincial Engineer) for the details of the work on the Sander's Brook drainage scheme; and to Mr. R. T. Jones for permission to use the photographs.

It should also be mentioned that this scheme was commenced under the care of the Assistant Director of Public Works (Mr. O. G. Price), who was former Public Works Engineer.

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EXPLANATION OF PLATE I

- Fig. 1. The upper part of Sander's Brook before permanent canalization.
- Fig. 2. The upper part of Sander's Brook after permanent canalization.



Fig. 1



Fig. 2



EXPLANATION OF PLATE II

- Fig. 1. Part of a street in area B before permanent drainage.
- Fig. 2. Part of a street in area B after permanent drainage.



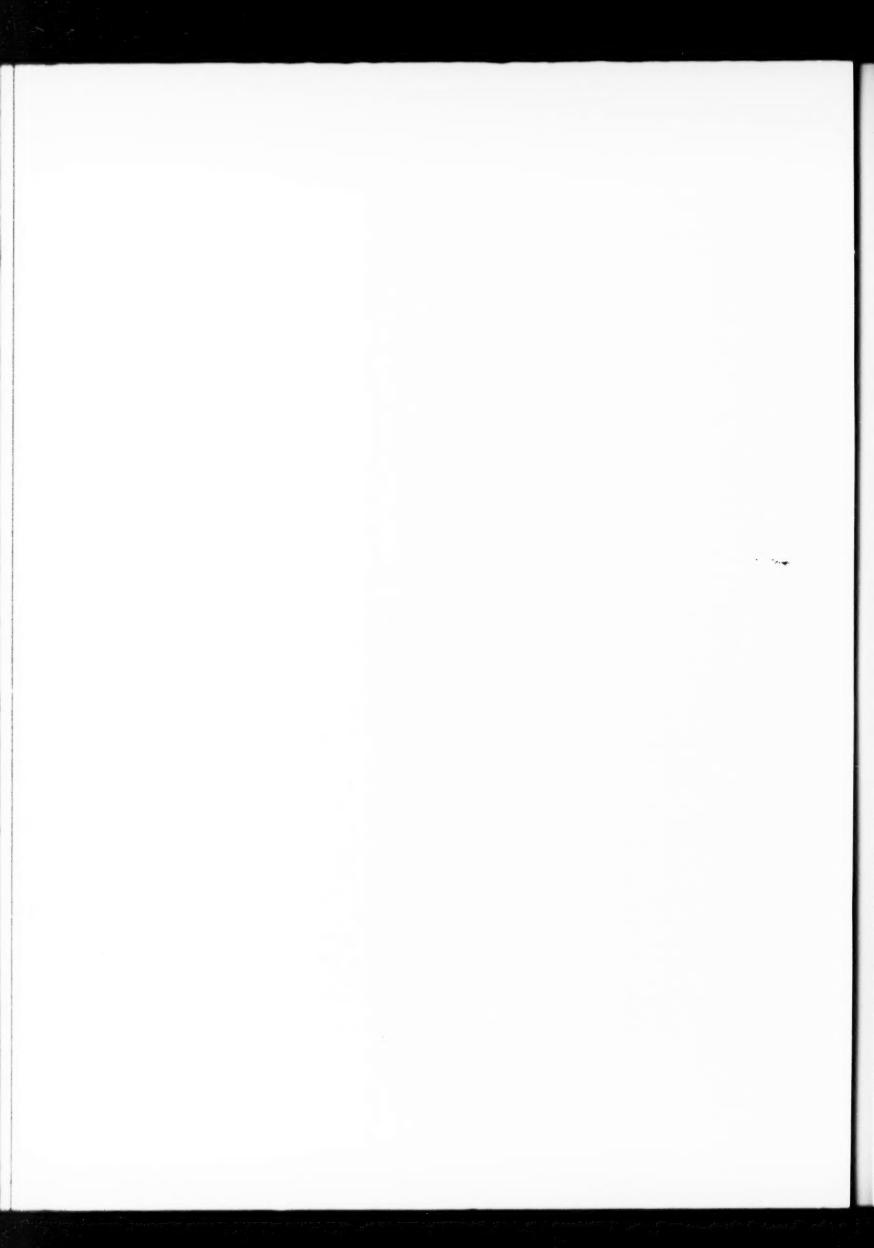
Fig. 1



Fig. 2

H. R. Grubb, Ltd., Poplar Walk, Croydon





ON A COLLECTION OF CESTODA FROM THE BELGIAN CONGO

BY

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AND

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WITH AN INTRODUCTION BY

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(Received for publication February 1st, 1939)

PART I

INTRODUCTION

By J. Schwetz

In a scientific expedition to the Belgian Congo undertaken in 1936, my programme included, amongst other things, the study of blood parasites in various animals. I took advantage of this opportunity to examine the same animals for helminths. Such a collection seemed to me of much interest, as the Congo helminths have not yet been studied at any length.

During my six months' stay in different parts of the Lower Congo and the Kwango, I collected a fairly large amount of material from various birds, antelopes, monkeys, rodents, lizards, serpents and fish.

Unfortunately, I was only able to bring back to Europe a small portion of my findings in a condition sufficiently good for them thoroughly to be examined and determined. My very rudimentary equipment and, more especially, the shortage of adequate containers were the chief cause of the deterioration of the major part of my material.

For these reasons, my helminthological survey was necessarily incomplete, the collection comprising almost exclusively nematodes and cestodes, i.e., it contained scarcely any trematodes.

In this work we deal only with the identity and classification of cestodes, and only with those which were in a fit condition to be taken to the Liverpool School of Tropical Medicine and to be studied under the direction of Dr. Southwell. We include, however, identifications on one Acanthocephala and two linguatulids found amongst the Cestoda.

My collection was made in the following localities:

I. Lower Congo: Banana, Malela, Tshela, Boma and Thysville.

II. Kwango River: Popokabaka, Kasongo Lunda (Kasanga), Chutes François-Josephe and Chutes Guillaume.

Most of the cestodes collected were from rodents, reptiles and birds, of which the following notes give details.

I. RODENTS

Whereas in the Lower Congo the domestic rat belongs to the species *Rattus rattus* (*frugivorus*), that of the Kwango is *Mastomys coucha*. Moreover, in the Kwango district, in addition to *M. coucha* I discovered ten other species of wild rats and mice. Out of these ten species we found cestodes in six (see below).

We may briefly mention here that the various wild rodents were found to be much less infected with worms than the domestic ones. In the two species *Rattus rattus* and *Mastomys coucha* cestodes were found in the intestine, whereas nematodes occurred in the stomach, sometimes in large quantities (*Protospirura muricola*).

As to other mammals, we found cestodes in several squirrels (Funisciuris congicus) from Thysville, and once in a monkey (Cercopithecus neglectus) from the shores of the Kwango River.

II. COLD-BLOODED ANIMALS (Lizards and snakes)

Cestodes were obtained from the lizards and snakes noted in the list given later in this paper. In several specimens of lizards belonging to two other genera (Mabuia maculilabris and Agama colonorum), we found various nematodes but no cestodes.

We also examined a large number of various fishes, but, with two exceptions, found only nematodes. The two exceptions were a *Clarias* sp. (Kwango River), which contained numerous Cestodaria (*Lytocestus*), and a young hammer-shark (Banana-mouth of the Congo River), which contained numerous small Lecanicephalidae.

III. BIRDS

About 500 birds were shot and examined, 250 on the shores of the Kwango River, 130 at Thysville, and the remainder at Boma and Banana.

At least 100 were found to contain cestodes, but, for reasons explained above, only about half of them were well preserved.

Of all the birds examined, the most heavily infected were guinea-fowls (Numidae; *Numida* and *Guttera*), which were parasitized by hundreds of cestodes. In most of the other infected birds we found only a few worms, in some cases only one or two. Nematodes were very rare.

PART H

BY T. SOUTHWELL AND F. LAKE

The following is a list of the parasites identified and the hosts from which they were obtained:

Parasite	Host
PHYLUM PLATYHELMINTHES CLASS CESTODA SUPERFAMILY I. Taenioidea ZWICKE, 1841 FAMILY 1. TAENIIDAE LUDWIG, 1886 Genus I. Taenia Linnaeus, 1758 Taenia taeniaeformis (Batsch, 1786) Wolffhügel, 1911 Larval form only, Cysticercus fasciolaris	Rattus rattus
Genus II. Catenotaenia Janicki, 1904 Catenotaenia pusilla (Goeze, 1782) Janicki, 1904 Catenotaenia lobata Baer, 1925	
Family 2. ANOPLOCEPHALIDAE Cholodkovsk Subfamily I. Anoplocephalinae Fuhrmann, 1907 Genus I. <i>Moniezia</i> R. Blanchard, 1891 <i>Moniezia ?carrinoi</i> (Diamare, 1900) Fuhrmann, 1902	
Genus II. Bertiella Stiles and Hassall, 1902 Bertiella studeri (R. Blanchard, 1891) R. Blanchard, 1891 Bertiella pinguis (Fuhrmann, 1904) Stiles and Hassall, 1902	-
Subfamily II. Linstowinae Fuhrmann, 1907 Genus I. Zschokkeella Ransom, 1909 Zschokkeella guineensis (Graham, 1908) Ransom, 1909	Centropus superciliosus loandae
Genus II. Oochoristica Luehe, 1898 Oochoristica agamae Baylis, 1919	Psammophis brevirostris Psammophis sibilans Chamaeleon etienii Gerrhosaurus flavigularis nigrolineatus

FAMILY 3. DAVAINEIDAE FUHRMAN	N, 1907		
SUBFAMILY I. DAVAINEINAE BRAUN, 190	,		
Genus Raillietina Fuhrmann, 1920			
Raillietina (R.) trapezoides Janicki, 1904			Arvicanthis striatus
Raillietina (R.) gracilis Janicki, 1904			
Raillietina (R.) echinobothrida (Mégnin,			
Blanchard, 1891			Numida sp.
Raillietina (R.) undulata Fuhrmann, 1909			
Raillietina (R.) tetragona (Molin, 1858) R.			
1891			Gallus gallus
Raillietina (R.) madagascariensis (Dav.,			0
Blanchard, 1891			Mastomys coucha
Raillietina (R.) permista sp. nov			
1			permista
Raillietina (F.) korkei Joyeux and Houder	ner, 192	7	
Raillietina (P.) bomensis sp. nov			
()			friedmanni
Raillietina spp			9
11			Campethera caroli caroli
SUBFAMILY II. IDIOGENINAE FUHRMANN	, 1907		
Genus <i>Idiogenes</i> Krabbe, 1868			
Idiogenes otidis Krabbe, 1868			Lyssotis melanogaster
Idiogenes flagellum (Goeze, 1782) Cholodk	ov., 190	5	Milvus tenebrosus
			aegyptius
E A HWMENOLEDIDIDAE D.			1000
FAMILY 4. HYMENOLEPIDIDAE RA			HENRY, 1909
SUBFAMILY I. HYMENOLEPIDINAE RANSO	м, 1908	,	
Genus I. Hymenolepis Weinland, 1858	D1 1		
Hymenolepis diminuta (Rudolphi, 1819) R.	Blancha		D
1891	• •		Rattus rattus
			Pelomys frater
			Steatomys pratensis
Hymenolepis fraterna Stiles, 1906			Rattus rattus
Hymenolepis spinosa von Linstow, 1906			Rostratula benghalensis
Hymenolepis stylosa (Rudolphi, 1810) Vol	z, 1899	• •	Balopogon indicator indicator
Hymenolepis unilateralis (Rudolphi, 1819)	Fuhrma	ınn,	
1906			Butorides striatus
			atricapillus
			•

PARASITE Host ? Hymenolepis fringillarum (Rudolphi, 1809) Fuhrmann, 1906 .. Dicrurus modestus coracinus Hymenolepis varicanthos sp. nov. ... Ibis ibis Hymenolepis spp. .. Lophuromys sp. Vinago calva calva Melanobucco minor intercedens Genus II. Echinocotyle R. Blanchard, 1891 Echinocotyle rosseteri R. Blanchard, 1891 Spermaspiza haematina pustulata Genus III. Haploparaxis Clerc, 1903 Haploparaxis crassirostris (Krabbe, 1809) Clerc, 1903 Galachrysia nuchalis nuchalis Genus IV. Oligorchis Fuhrmann, 1906 .. Galachrysia nuchalis . . nuchalis SUBFAMILY II. DILEPININAE FUHRMANN, 1907 Genus I. Dilepis Weinland, 1858 Dilepis irregularis sp. nov. .. Rostratula benghalensis Genus II. Echinorhynchotaenia Fuhrmann, 1909 Echinorhynchotaenia tritesticulata Fuhrmann, 1909 . . Anhinga rufa rufa SUBFAMILY III. PARUTERININAE RANSOM, 1909 Genus I. Anonchotaenia Cohn, 1900 Anonchotaenia bobica Clerc, 1903 Tshagra senegala rufofusca Genus II. Biuterina Fuhrmann, 1902 Biuterina meropina (Krabbe, 1869) var. macrancistrota Dryoscopus angolensis angolensis Merops nubicoides Biuterina cylindrica Fuhrmann, 1908 Astimastillas falhensteini Biuterina spp. Pycnonotus tricolor . . tricolor Caprimulgus fossii welwitschii

PARASITE			Host
Genus III. Metroliasthes Ransom, 190	00		
Metroliasthes lucida Ransom, 1900			Numida sp. Gallus gallus Guttera edouardi
FAMILY 5. PROTEOCEPHALIDAE Genus <i>Ophiotaenia</i> La Rue, 1911	LA RUE,	1911	Success cuonaras
Ophiotaenia punica (Cholodkovsky, 1908) La Rue,	1911	Causus rhombeatus
0 1:	• •		Boodon olivaceus Boodon lineatus Undetermined snakes
Superfamily II. Dibothriocephaloides	STILES	1906	Chacterininea shakes
FAMILY DIBOTHRIOCEPHALIDAE			
Genus I. Bothridium Blainville, 1824	,		
Bothridium pithonis Blainville, 1824			Python sebae
Genus II. Duthiersia Perrier, 1873			
Duthiersia elegans Perrier, 1873			Varanus niloticus
Genus III. Lytocestus Cohn, 1908			
Lytocestus adhaerens Cohn, 1908			Clarias sp.
Superfamily III. Lecanicephaloidea	Southwe	LL, 19	030
FAMILY LECANICEPHALIDAE BRA	un, 1900		
Genus Lecanicephalum Linton, 1890			
Lecanicephalum peltatum Linton, 1890		• •	Zygaena malleus
PHYLUM NEMATHELMINTHES VO		-	Carus, 1863)
CLASS ACANTHOCEPHALA RUDOLPHI,			
FAMILY GIGANTHORHYNCHIDAE	HAMANN	v, 189	2
Genus Empodius Travassos, 1916			
Empodius taeniatus (von Linstow, 1901)	• •		Numida sp. Guttera edouardi
PHYLUM ARTHROPODA			
CLASS PENTASTOMIDA			
FAMILY LINGUATULIDAE SHIPLEY			
SUBFAMILY POROCEPHALINAE SAMBON,			
Genus I. Porocephalus Humboldt, 1811		010	D
Porocephalus clavatus (Wyman, 1845) S	ambon, I	910	Psammophis notostictus
Genus II. Armillifer Sambon, 1922 Armillifer armillatus (Wyman, 1847) Sa	ambon, 1	922	Python sebae

PHYLUM PLATYHELMINTHES

CLASS CESTODA

Superfamily I. Taenioidea Zwicke, 1841

Synonym: ORDER CYCLOPHYLLIDEA BRAUN, 1900

FAMILY 1. TAENIIDAE LUDWIG, 1886

Genus I. Taenia Linnaeus, 1758

Taenia taeniaeformis (Batsch, 1786) Wolffhügel, 1911

The larval form of this species, namely Cysticercus fasciolaris, was found in the liver of Rattus rattus.

Genus II. Catenotaenia Janicki, 1904

Synonym: Cladotaenia Cohn, 1901

In this genus the scolex is unarmed and a rostellum is absent. The mature and gravid segments are much longer than broad. The genital pores are irregularly alternate. The testes are numerous and situated posterior to the ovary. The uterus consists of a central stem and a number of compound lateral branches, except perhaps in ? C. symmetrica Baylis, 1927. Worms of this genus are for the most part parasitic in rodents.

Catenotaenia pusilla (Goeze, 1782) Janicki, 1904

Several specimens from the intestine of Mastomys coucha; Kwango.

The worms measured up to 10 cm. in length and had a maximum breadth of 1.5 cm. The excretory system is not a network, but consists of two lateral tubes on each side, one dorsal and one ventral. The uterus is a central stem with about 12 lateral branches on each side.

The species has been recorded from Mus musculus, Rattus rattus, Rattus norvegicus, Apodemus sylvaticus, Glis glis, Evotomys glareolus and Microtus arvalis.

Catenotaenia lobata Baer, 1925

A number of specimens from the intestine of Funisciurus congicus; Kwango. Baylis (1927)* gave a revision of the genus Catenotaenia, which contained four species only. In two of these species, namely C. pusilla and C. dendritica, the excretory system is of the normal type. In the other two species, C. lobata and C. symmetrica, the excretory system consists of a network. One of the main differences between the two latter species is the fact that in the former the uterus consists of a central stem with about 20 lateral branches on each side, whereas in the latter no definite uterus was observed. The eggs lie singly in the parenchyma.

^{*} A full list of references will appear at the end of the second part of this paper, which will be published in the next number of this journal.

In our specimens the excretory system is undoubtedly a well-defined network, and the uterus is a central stem with about 18 lateral branches on each side. We therefore refer our worms to the species *lobata*.

We would point out, however, that, while *C. lobata* was recorded by Baer (1925) from a rat in the Belgian Congo, our specimen was taken from a squirrel found in the same locality.

Aparently the only species of this genus previously recorded from a squirrel is C. dendritica Riggenbach, 1895.

Family 2. ANOPLOCEPHALIDAE CHOLODKOVSKY, 1902 Subfamily I. Anoplocephalinae Fuhrmann, 1907 Genus I. *Moniezia* R. Blanchard, 1891

Moniezia?carrinoi (Diamare, 1900) Fuhrmann, 1902 Synonyms: Taenia trichoglossi von Linstow, 1888 Paronia carrinoi Diamare, 1900

Several specimens from the intestine of *Pycnonotus tricolor* ; Thysville.

The worms were about 3 cm. in length, with a maximum breadth of 2 mm. Those examined were gravid, but the eggs were not fully developed.

The head is unarmed; the genital organs are double in each segment, as are also the genital pores. The uteri arise, one on either side, from the oviduct, and extend in the median direction. The vagina and cirrus pouch on both sides pass dorsal to both excretory vessels.

We have had great difficulty in relegating these worms to any particular species, being aware of the fact that *M. carrinoi* was described from an Australian parrot, whereas our species is from a passerine bird from the Belgian Congo. We have carefully studied Fuhrmann's paper of 1902, and the division which he makes of the original material into two species leaves us in very considerable doubt as to the distinctiveness of the two. In the same paper Fuhrmann erects a third species, *M. ambigua*, obtained from a South American parrot. It appears to us almost impossible to differentiate these three species from one another.

We have therefore, with some uncertainty, identified our species as M. carrinoi (Diamare, 1900) Fuhrmann, 1902.

Genus II. Bertiella Stiles and Hassall, 1902

Bertiella studeri (R. Blanchard, 1891) R. Blanchard, 1891 Synonyms: Bertia satyri Blanchard, 1891 Bertia polyorchis von Linstow, 1905 Bertiella cercopithecei Beddard, 1911; etc.

A single specimen from the intestine of *Cercopithecus neglectus*; it measured about 20 cm. in length and had a maximum breadth of 1 cm. All the segments are very much broader than long. The genital pores are irregularly alternate.

There are a large number of testes. The ovary is asymmetrical, being situated on the poral side of the segment. The vitelline gland is extraordinarily minute. The uterus is a transverse lobulated sac not extending lateral to the excretory vessels. The eggs bear a typical pyriform apparatus.

This species has been recorded several times from man in Mauritius and once in India. It has also been recorded from Hylobates hurlock, Cercopithecus

pygerythrus, Cynmologus sinicus and C. fascicularis.

Bertiella pinguis (Fuhrmann, 1904) Stiles and Hassall, 1902 Synonyms: Bertia pinguis (von Siebold) Fuhrmann, 1904 Anoplocephala pinguis (Fuhrmann, 1904) Fuhrmann, 1921 Ophryocotyloides pinguis (Fuhrmann, 1904) Baer, 1927

Two specimens from the intestine of *Bycanistes* sp.; Kwango. They measured about 40 cm. in length and about 4 mm. in breadth.

Fuhrmann (1904) first described this species from a bird (Bucorax abyssinicus) from West Africa. Later, the species was referred to the genus Bertiella Stiles and Hassall, 1902. In 1921 Fuhrmann redescribed the species and referred it to the genus Anoplocephala.

Baer (1927) restricted the genus *Bertiella* to two species found in primates, and placed all the remaining species in a new genus, which he named *Prototaenia*,

the adult forms of which are found in marsupials and insectivores.

The single species found in birds, namely *B. pinguis*, was referred by Baer to the genus *Ophryocotyloides* Fuhrmann, 1920. Baer stated that, on re-examining the scolex of this species, he found it to be armed with a double crown of from 150 to 200 hooks of the typical *Davainea* type, each measuring from 5μ to 7μ in length.

Having Baer's observation in mind, we examined the scolex in our worm under the oil-immersion with meticulous care, but were unable to find any trace either of a rostellum or of hooks. In view of this fact we have no option

but to retain the worm within the genus Bertiella.

Baylis (1934a), dealing with two new species of the cestode genus *Bertiella*, is inclined to the view that the genus *Prototaenia* cannot at present be distinguished from *Bertiella*, and he prefers to regard it as a synonym of the later genus.

Subfamily II. Linstowinae Fuhrmann, 1907 Genus I. Zschokkeella Ransom, 1909

Zschokkeella guineensis (Graham, 1908) Ransom, 1909

Several fragments, one with a head, from the intestine of Centropus superciliosus loandae; Boma. They are doubtfully referred to the above species.

The head is unarmed and bears four suckers. The genital pores are unilateral.

Details of a mature segment could not be ascertained, but it was noted that the ovary was placed asymmetrically. The uterus consists of a large number of

capsules, each apparently containing three or four eggs.

Clearly the species belongs to the genus Zschokkeella, but the specific identity of the parasite is somewhat doubtful. Z. guineensis has been obtained from Cricetomys gambianum; Z. linstowi (Parona, 1885) from Numida ptilorhyncha; Z. muricola Baylis, 1920, from Epimys rattus; and Z. remota von Linstow, 1905, from Cercopithecus pyrrhonotus.

As far as the present authors are aware, no species of this genus has hitherto been recorded from Coccygiformes, and it is unfortunate that, owing to lack of material, the specific identity of the parasite from this bird cannot definitely

be determined.

Genus II. Oochoristica Luehe, 1898

Oochoristica agamae Baylis, 1919

Several specimens from the intestine of *Psammophis brevirostris*, *P. sibilans*, *Chamaeleon etienii* from Banana and Popokabaka, and *Gerrhosaurus flavigularis nigrolineatus* from Kwango.

The worms measure up to 6 cm. in length, and the maximum breadth is 1.6 mm. The scolex has a breadth of 1 mm. and the diameter of the suckers is from 240μ to 300μ . All the mature segments are broader than long, the gravid ones being square or even longer than they are broad. The genital pores are irregularly alternate, and are situated in the anterior third of the segment.

The testes are oval bodies, rather irregular in shape, from 18 to 24 being on the poral side and 20 to 26 aporally. They may be situated either posterior to the ovary or posterior and lateral to it. The cirrus sac extends about one-third of the breadth of the segment and is extremely narrow, having a breadth of

only 18μ .

The ovary is bilobed, shortened antero-posteriorly, and situated slightly on the poral side of the segment. The eggs lie singly in the parenchyma; they are about 30μ in diameter, containing a spherical hexacanth embryo with a diameter of 26μ .

In 1934 Meggitt published a table showing the characters of the known species of the genus *Oochoristica*. We are unable to differentiate our species from *Oochoristica agamae* Baylis, 1919, which Meggitt considers to be a synonym of *O. ameivae* (Beddard, 1914).

The morphological differences between many of the species given in Meggitt's table seem so small and trivial that it is easy to believe with Meggitt that they may be normal variations within the limits of a single species.

A strobila containing both mature and gravid segments, but without scolex, was found in a phial containing parasites from *Rana occipitalis* Boma; they were indistinguishable from those of *O. agamae* Baylis, 1919. It is possible that

a mistake may have been made with reference to the host, but we see no reason for assuming such to be the case.

Another specimen, very badly preserved, and indistinguishable from

(). agamae Baylis, 1919, was obtained from Gastropyxis smaragdina.

We call attention to the fact that *O. agamae* in our collection was obtained from snakes, lizards, chameleons and possibly from frogs. The occurrence, if such is actually the case, of the same parasite in such a wide range of hosts is, we think, quite unusual.

Family 3. DAVAINEIDAE FUHRMANN, 1907 SUBFAMILY I. DAVAINEINAE BRAUN, 1900 Genus *Raillietina* Fuhrmann, 1920

Raillietina (Raillietina) trapezoides Janicki, 1904

Several specimens, only two with heads, from the intestine of Arvicanthis striatus; Kwango.

The worms measure 5 or 6 cm. in length and have a maximum breadth of about 1.2 mm. All the segments are broader than long, and the genital pores are unilateral.

The rostellum is armed with about 140 hammer-shaped hooks, each measuring about 9μ . The suckers are also armed with several marginal rows of hooks. There are about 15 testes, situated internal to the lateral water-vessels. The cirrus sac extends slightly median to the excretory vessel. The uterus consists of a comparatively small number of capsules, each containing not more than 12 eggs.

The species has previously been recorded from Mus variegatus in Egypt.

Raillietina (R.) gracilis Janicki, 1904

A few specimens of this species were obtained from the intestine of Thryonomys swinderianus.

The worms measure about 9 cm. in length and have a maximum breadth of about 4 mm. The strobila tends to become gross and fleshy. All the segments are broader than long, except for a few of the posterior ones which are almost square and somewhat barrel-shaped. The genital pores are unilateral, and are situated near the anterior lateral margin of the segment. The rostellum is armed with about 120 hooks arranged in two rows, each hook measuring about 40μ . The periphery of each sucker is also armed with a few rows of hooks. Each uterine capsule contains several eggs. Janicki obtained this species from the intestine of *Mus flavides* at El Tor in the U.S.A..

The following is a list of species of Davaineidae which have been recorded from rodents:

- 1. Raillietina (Paroniella) blanchardi Parona, 1898.
- 2. Raillietina (Raillietina) celebensis Janicki, 1902.

- 3. Raillietina (?—) fluxa Meggitt and Subramanian, 1927.
- 4. Raillietina (?--) funebris Meggitt and Subramanian, 1927.
- 5. Raillietina (R.) gracilis Janicki, 1904.
- 6. Raillietina (?--) isomydis (Setti, 1892) Parona, 1900.
- 7. Raillietina (R.) madagascariensis (Dav., 1870) R. Blanchard, 1891.
- 8. Raillietina polycalceola (= Davaineoides) Janicki, 1902.
- 9. Raillietina (P.) retractilis Stiles, 1895.
- 10. Raillietina (Fuhrmanetta) salmonis Stiles, 1895.
- 11. Raillietina (R.) trapezoides Janicki, 1904.
- 12. Raillietina sp. Douthitt, 1915.
- 13. Raillietina sp. Johnston, 1918.

Of the above species the only ones in which the pores are unilateral, the suckers armed, and of which each uterine capsule contains many eggs are the following: (a) Raillietina (R.) trapezoides Janicki, 1904. In this species there are 160 rostellar hooks, each measuring 8μ . (b) Raillietina (R.) gracilis Janicki, The rostellum is armed with 120 hooks in a single row, each hook measuring 47μ . The suckers are armed, and the genital pores are unilateral. Fuhrmann (1920), however, states that the pores are irregularly alternate. The number of eggs in a capsule is not known. (c) Raillietina (R.) madagascariensis (Dav., 1870) R. Blanchard, 1891. In this species the genital pores are unilateral and the suckers are armed. There are 90 hooks, arranged in a double row, each hook measuring 18μ in length. Fuhrmann (1920) states that each uterine capsule contains many eggs, but according to Meggitt (1921a) each capsule contains only a single egg (see below).

It will be seen, therefore, that our species differs from *trapezoides*, amongst other things, in the size of the hooks, and from *madagascariensis* in the number of hooks. The species closely resembles *gracilis*, but appears to differ slightly in the size of the hooks, which we consider to be a variation within the limits of the species.

Raillietina (R.) echinobothrida (Mégnin, 1880) R. Blanchard, 1891

A large number of specimens from the intestine of Numida sp. from Kwango. Stiles (1896) states that in R. tetragona the rostellum bears 200 hooks, each 6μ in length, the genital pores being generally unilateral, occasionally alternate; whilst in R. echinobothrida the rostellum bears 100 hooks, each 8μ in length, the genital pores being alternate. Ransom (1905), however, points out that in R. tetragona the rostellum bears about 100 hooks, each $6-8\mu$ long, and that the genital pores are unilateral; while in R. echinobothrida the rostellum bears 200 hooks, each $10-13\mu$ in length, the genital pores being irregularly alternate and, rarely, almost unilateral. Finally, Tubangui (1927) states that the hooks on the rostellum and suckers of R. echinobothrida are larger than those of R. tetragona, the former being $10-13\mu$ in length and the latter $6-8\mu$. He also remarks that in R. tetragona the rostellar hooks number about 100 and are arranged in a single row.

It will thus be seen that there is some confusion regarding the identity of these two species, but that all investigators agree that the hooks in *R. echino-bothrida* are larger than those in *R. tetragona*.

In our specimens the rostellum was armed with about 200 hooks, each measuring about 12μ . The suckers were powerfully armed marginally with several rows of large hooks. The genital pores, however, were unilateral and situated a little in front of the middle of the lateral margin of the segment. The eggs are in capsules, with at least 12 eggs in each capsule.

On account of the number and size of the hooks on the rostellum, and of the powerfully armed suckers, we refer our worms to the species R. echino-bothrida.

Raillietina (R.) undulata Fuhrmann, 1909

A large number of specimens of this species were obtained from Corytheola cristata from Kwango.

They measure 6 or 7 cm. in length and have a maximum breadth of 1.5 mm. The pores are unilateral and situated a little anterior to the middle of the segment. All the segments are much broader than long, except the last three or four, which are fully gravid. The posterior margin of each segment overlaps the anterior margin of the succeeding one in a bell-like manner.

Head. The rostellum is armed with about 180 hammer-shaped hooks, each measuring from 20μ to 26μ , usually about 25μ . The periphery of the suckers is armed with 5 or 6 rows of hooks.

Male genital organs. There are about 10 or 12 testes on each side of the ovary. The cirrus pouch is curved, and extends only to the excretory vessels.

Female genital organs. The ovary is very small, fan-shaped, and situated in the centre of the segment, posteriorly. The vitelline gland is also very small, and is placed behind the ovary. The egg capsules each contain several eggs (8 or 9), and do not extend lateral to the water-vessel.

Fuhrmann (1909b) obtained his species from Corytheola cristata in West and Central Africa. In his original description of this parasite he did not mention the presence of spines on the suckers. His specimens were probably not gravid, as he does not describe the uterus. Our specimens were obtained from the same host and from about the same area, and there is no doubt as to the identity of the species.

Raillietina (R.) tetragona (Molin, 1858) R. Blanchard, 1891

A large number of specimens from the intestine of Gallus gallus; Kwango. The worms measure up to 10 cm. in length and have a maximum breadth of about 2.5 mm. All the segments are broader than long, except for a few of the posterior ones, which tend to become campanulate. The genital pores are unilateral.

The rostellum is armed with a double crown of about 100 hammer-shaped hooks, each measuring about 7μ . Each sucker carries about 10 rows of hooks round its margin.

The egg capsules extend lateral to the excretory vessels, and each capsule contains a number of eggs.

Raillietina (R.) madagascariensis (Dav., 1870) R. Blanchard, 1891

A few specimens from the intestine of *Mastomys coucha*; Kwango. The worms measure about 6 cm. in length and have a maximum breadth of about 1.8 mm. The genital pores are unilateral, and are situated midway on the lateral margin of the segment. All the segments are broader than long, except for a few of the posterior gravid ones, which are a little longer than broad and somewhat barrel-shaped.

The rostellum bears about 70 hooks arranged in a single crown, each hook measuring about 12μ in length. The margins of the suckers are armed with several rows of extremely minute hooks, which can only be seen under an oil-immersion lens.

Each mature segment contains from 18 to 22 testes, situated on each side of, but not posterior to, the ovary. In each segment the greater number of testes lie on the aporal side. The ovary and vagina present no distinctive characters.

In each gravid segment the uterus consists of about 70 capsules, which extend lateral to the excretory vessels on both sides. Each capsule contains many eggs.

Diagnosis. In the description of Raillietina (R.) gracilis (see above) we gave a list of the species of Raillietina which have been recorded from rodents. We further discussed those species in which (1) the pores are unilateral, (2) the suckers are armed, and (3) those in which each uterine capsule contains many eggs.

Our species most closely approximates to R. madagascariensis, in which the rostellum bears 90 hooks arranged in a double row, each hook measuring 18μ . In our species the rostellum bears from 65 to 70 hooks, arranged in a single row, and each hook measures $12-13\mu$.

We are of opinion that such small differences as these may be normal variations for this species in the rat, and we therefore do not propose to erect a new species.

Raillietina (R.) permista sp. nov.

Several worms, of which there was only one with a scolex, were obtained from the intestine of Campethera permista permista from Thysville. The largest worm measured about 3 cm. in length and had a maximum breadth of about 1.5 mm. The posterior segments measure 2 mm. in length and 1 mm. in breadth. The lateral posterior margins of each segment overhang the anterior part of the following segment. The genital pores are unilateral, and are situated in the anterior third of the lateral margin.

Head. The scolex measures 250μ in length and 240μ in breadth. Each sucker, which has a diameter of 136μ , is armed peripherally with a few rows of spines. The rostellum bears about 36 hammer-shaped hooks arranged in two rows, each hook measuring 18μ . The cuticle surrounding the rostellum is covered with extremely minute spinules, only visible under the oil-immersion.

The neck is very short, not longer than the head.

Male genital organs. There are from 15 to 20 testes, placed posteriorly and laterally to the ovary. The cirrus pouch is small, not reaching the excretory vessel. The seminal vesicle is large and prominent, and is situated near the middle of the segment.

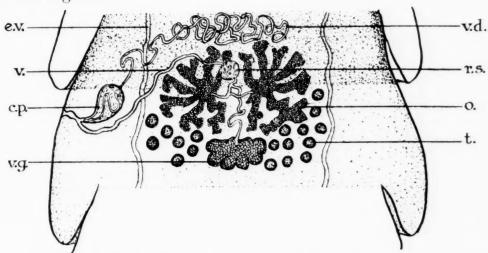


Fig. 1. Raillietina (R.) permista sp.nov. Mature segment (> 95).

EXPLANATION OF LETTERING TO ALL FIGS.

C.,	cirrus.	0.,	ovary.
c.p.,	cirrus pouch.	r.s.,	receptaculum seminis.
c.m.,	circular muscles.	s.a.,	sacculus accessorius.
d.v.,	dorsal excretory vessels.	8.2.,	shell gland.
e.l.m.,	external longitudinal muscles.	s.v.,	seminal vesicle.
e.s.v.,	external seminal vesicle.	t.,	testes.
e.r.,	excretory vessels.	и.,	uterus.
h.,	hooks.	7,	vagina.
i.l.m.,	internal longitudinal muscles.	v.d.,	vas deferens.
i.s.r.,	internal seminal vesicle.	v.g.,	vitelline gland.
n.,	nerve.	v.v.,	ventral excretory vessels.

Female genital organs. The ovary is median and bilobed, each lobe being roughly fan-shaped. The vitelline gland is large and lobular, and is situated posterior to the ovary (fig. 1).

Uterus. This splits up into at least 50 capsules, each containing about six eggs. The capsules do not extend lateral to the excretory vessels, although in a few segments what appeared to be small clusters of degenerated eggs were noted lateral to the vessel.

As far as we are aware, the only species of the genus *Raillietina* in which the rostellum bears less than 50 hooks are:

1. R. campanulata Fuhrmann, 1909. In this species the rostellum bears 40-42 hooks, each hook measuring 27μ . The suckers are unarmed, and the

genital pores are irregularly alternate. The worm was obtained from Galliformes in Brazil.

2. R. oligocantha Fuhrmann, 1909. In this species the rostellum bears 34 hooks, each of which measure from $21-23\mu$. The suckers are unarmed, and the genital pores are irregularly alternate. The species was obtained from Rhynchotus rufescens in South Brazil.

Our species differs from both these, not only in the size and number of hooks on the rostellum, but in the fact that the suckers are armed and the genital pores are unilateral.

Raillietina (Fuhrmannetta) korkei Joyeux and Houdemer, 1927

Five large fragments of strobila, one with a scolex, from the intestine of *Columba livia*; Boma.

It would appear that the fully developed worm measures probably 30 cm. in length and has a maximum breadth of 3 mm. The segments are broader than long, except for the gravid ones, which are longer than broad. The genital pores are irregularly alternate and situated in the anterior half of the lateral margin of the segment.

Each of the four suckers is armed round the margin with about 10 rows of relatively large spines arranged diagonally.

The rostellum bears 120 hooks arranged in a double row. The hooks in the anterior row measure 20μ and those in the posterior 17μ .

It was not possible accurately to count the number of testes, but there appeared to be about 30, the greater number being on the aporal side. A few were situated behind the ovary. The cirrus pouch is pyriform and does not extend to the excretory vessel. The vas deferens is closely coiled and in many segments can be seen extending nearly to the middle of the segment.

The ovary is bilobed; posterior to it is a rather prominent, deeply staining, vitelline gland. The thick-walled vagina lies posterior to the cirrus pouch and the vas deferens; in mature segments it dilates near the pore into a prominent receptaculum seminis.

The eggs are in groups of from 4 to 8 in each capsule, which extend, here and there, lateral to the excretory vessels.

Diagnosis. The armed suckers, the number and size of the rostellar hooks, the irregularly alternate genital pores, and the disposition of the eggs in capsules leave no room for doubt as to the identity of this parasite.

The species has previously been recorded from pigeons in India. As far as we are aware this is the first record of it in Africa.

In the original description of this species by Joyeux and Houdemer (1927), the number of hooks on the rostellum is said to be from 150 to 160, varying in length from 18μ to 20μ . In our specimen the number of hooks was quite definitely and exactly 120, the scolex offering excellent facilities for accurate counting. The variation in the number and size of the hooks is, we think, of little importance, as the general characters agree closely in every other detail.

Raillietina (Paroniella) bomensis sp. nov.

Three specimens, rather poorly preserved and very strongly contracted, from the intestine of *Melanobucco bidentatus friedmanni*; Boma.

The worm measures about 2 cm. in length, and has a maximum breadth of from 2 to 3 mm. The strobila is very stout and fleshy, owing to excessive contraction. The segments are all broader than long, except for the gravid ones, which are almost square. The genital pores are unilateral, and are situated at the anterior lateral corner of the segment.

Head. The rostellum bears about 200 hammer-shaped hooks arranged in a double row; each hook measures from 27μ to 30μ (fig. 2, A). The margin of each sucker bears about 7 rows of spines.

Male genital organs. The testes are situated in two fields, one on each side of the ovary. There are about 18 testes aporally and about 9 on the poral side. The cirrus pouch is pyriform and extends only about half way to the excretory vessel (fig. 2, B).

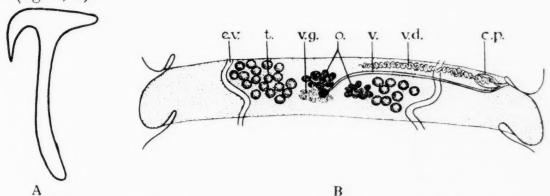


Fig. 2. Raillietina (P.) bomensis sp. nov. A.—Hook (× 1,300). B.—Mature segment (× 70).

Female genital organs. The ovary is situated in the middle of the segment. Owing to scarcity of material, details relating to the vagina were not noted. The uterus completely fills the gravid segments, extending laterally to the excretory vessels on each side. The capsules each contain a single egg. In none of our specimens was the uterus fully gravid.

Diagnosis. The large size of the hooks differentiates the species from all other Davaineinae which have uterine capsules containing a single oncosphere in each capsule. The species hitherto recorded from Piciformes are as follows:

- 1. R. cruciata Rudolphi, 1819. In this species there are 200 rostellar hooks measuring 14μ to 16μ , and the cirrus pouch extends almost to the aporal margin.
- 2. R. longispina Fuhrmann, 1909. In this species the suckers are unarmed. There are 300 rostellar hooks, each measuring from 14μ to 16μ .
- 3. R. rhynchota (Ransom, 1909). There are 400 rostellar hooks, measuring 18μ and 14μ .
- 4. R. frontina (Dujardin, 1845) R. Blanchard, 1891. The rostellum bears 175–200 hooks, each measuring from 8μ to 14μ , and each uterine capsule contains many eggs.

5. R. lutzi Parona, 1901. In this species the suckers are unarmed. Each uterine capsule contains two or more eggs. The head has not been described.

6. R. comitata (Ransom, 1909). The rostellum bears 80 hooks in a single row, each measuring about 12μ . Each uterine capsule contains from 6 to 12 eggs.

7. R. permista sp. nov. The rostellum bears 36 hooks, each measuring 18μ , and each uterine capsule contains about 6 eggs.

From all the species in which the uterine capsules contain a single egg, our species differs in the size of the hooks.

Baylis (1919a) described a species from *Haliaëtus vocifer* in Uganda, which he named *Davainea vaganda*, in which the rostellum is armed with numerous hooks, 25μ in length, arranged in two rows. The hooks on the suckers are very numerous, measuring 15μ in length. The pores are unilateral. The testes number 6–8.

Our species bears a fairly close resemblance to Baylis's species. It differs, however, in the number of testes and in the size of the hooks. The parasite which we have examined was immature, and a uterus was not present. Baylis's species was from a true sea-eagle (Accipitriformes); ours was from Piciformes.

Raillietina sp.

One fragment, with a head, from the intestine of Campethera caroli caroli; Thysville.

The segments were immature, and the four suckers were armed round their periphery with several rows of hooks. The rostellum bore a very large number of minute hammer-shaped hooks arranged in a double row, each hook measuring about 9μ .

Raillietina sp.

A few fragments, one with a head, from the intestine of *Nicator vires*; Thysville. The rostellum was armed with a double crown of about 300 hooks, each measuring about 8μ . The suckers were unarmed; no structural details could be observed.

Subfamily II. Idiogeninae Fuhrmann, 1907 Genus *Idiogenes* Krabbe, 1868

Idiogenes otidis Krabbe, 1868

Very numerous specimens from the intestine of Lyssotis melanogaster; Kwango.

Many of the worms measure about 7 cm. in length, but others are longer; the maximum breadth is about 1 mm. A large number of the segments are bell-shaped, being longer than broad. The genital pores are unilateral. In mature segments they are situated at the anterior lateral corner of the segment, but in gravid segments the pore is nearer to the middle of the lateral margin.

Head. The head is armed with about 70 hammer-shaped hooks arranged in a double row. Each hook measures 15μ or 16μ .

Male genital organs. There are from 15 to 22 testes situated posterior and lateral to the ovary. The cirrus pouch extends almost three-quarters of the width of the segment, and is six times as long as broad. It runs obliquely from the pore to the anterior aporal margin of the segment. The cirrus is very long and strongly armed.

Female genital organs. The ovary is bilobed and situated near the centre of the segment. The vagina is a stout tube; near the pore it crosses the cirrus sac and opens slightly anterior to the vas deferens. Its internal wall is strongly

armed with spines.

The uterus first appears as a horseshoe-shaped sac enveloping the ovary. Later it becomes lobulated, and in front of it a prominent paruterine organ develops.

Diagnosis. This parasite has been recorded previously from Otis tarda,

Tetrax tetrax and Houbara undulata in Africa.

We would call attention to the fact that we had some difficulty in deciding whether this worm belonged to the genus *Idiogenes* or *Paruterina*. We are aware that the former is referred to the family Davaineidae and the latter to the family Hymenolepididae. Their morphological characters, however, are identical, except that in some species of *Paruterina* the genital pores are irregularly alternate, whereas in other species of the same genus, and in all species of *Idiogenes*, the pores are unilateral. We are also aware that the shape of the hook in species of these two genera is very different. Nevertheless, we are of the opinion that these minute differences do not justify the retention of both these genera.

Idiogenes flagellum (Goeze, 1782) Cholodkovsky, 1905 Synonym: Idiogenes mastigophora (Krabbe, 1879) Volz, 1900

A very large number of specimens of this species were obtained from the

intestine of Milvus tenebrosus aegyptius from Kwango.

The worms measure up to 5 cm. in length and 1 mm. in breadth. In our specimens the majority of the segments are almost square, though a few of the more posterior gravid ones are a little longer than broad. The genital pores are unilateral and situated slightly anterior to the middle.

Head. This is almost square and has a breadth of about 700μ . The rostellum is armed with about 170 hooks in a double crown. Those on the anterior row measure 11μ in length, and those of the posterior 9μ . The margin

of each sucker bears about 5 rows of minute hooks.

Male genital organs. There are about 10 testes, situated posteriorly behind the ovary. The cirrus pouch is very prominent, running to the anterior margin and extending three-quarters the breadth of the segment. Its length is about four times its breadth. The cirrus itself is very long, and has a thick wall covered externally with large numbers of minute spines.

Female genital organs. The ovary is bilobed and situated in the centre of the segment. Immediately posterior to it is a large, granular, vitelline gland.

The vagina runs posterior to the cirrus pouch, and dilates immediately in front of the ovary into a conspicuous globular receptaculum seminis. The uterus arises posteriorly as an irregular sac. A paruterine organ develops anterior to it, and into this the eggs pass.

Diagnosis. This worm has been recorded from various species of Milvus. It is a common parasite in M. korschun and M. aegyptius in Africa. It also occurs in M. milvus and M. melanotus in Europe, Asia and Japan.

FAMILY 4. HYMENOLEPIDIDAE RAILLIET AND HENRY, 1909 SUBFAMILY I. HYMENOLEPIDINAE RANSOM, 1909 Genus I. Hymenolepis Weinland, 1858

Hymenolepis diminuta (Rudolphi, 1819) R. Blanchard, 1891

Numerous specimens from the intestine of *Pelomys frater* and *Steatomys pratensis* from Kwango, and from that of *Rattus rattus* from Boma.

Two species of Hymenolepis occur fairly commonly in the intestine of rodents, namely H. diminuta and H. horrida (von Linstow, 1901). In the latter species the cirrus is armed and the eggs measure from 38μ to 56μ by 19μ to 24μ , whilst in the former the cirrus is unarmed and the eggs measure from 50μ to 86μ .

In our specimens the cirrus was unarmed and the eggs measured from 64μ to 76μ . We therefore consider our parasites as being specimens of *H. diminuta*. This is, we believe, the first record of this parasite in *Pelomys frater*.

Hymenolepis fraterna Stiles, 1906

A very few specimens, only one with a head, were obtained from the intestine of *Rattus rattus*; Boma.

Hymenolepis spinosa von Linstow, 1906

Over 100 specimens from the intestine of Rostratula benghalensis from Kwango. The worms measure up to 2 cm. in length and about 1.4 mm. in breadth. All the segments are broader than long, and the posterior lateral margin of one segment overlaps the succeeding one. The genital pores are unilateral, and, in immature segments, are situated at the extreme anterior corner and are hidden by the margin of the preceding segment. In the more mature and gravid segments the pores can clearly be seen at the anterior margin of the segment.

Fig. 3.

Hymenolepis spinosa.

Hooks (× 1,100).

Head. This measures 180μ in length and 240μ in breadth. The rostellum is armed with 10 hooks, each of which measure about 30μ . They have the shape shown in fig. 3.

Male genital organs. There are three large testes, two being aporal and one poral. The cirrus pouch is large, extending across to the median margin of the aporal testes. The vesicula seminalis is enormous. It measures 111μ by 220μ , and is situated anteriorly in the middle of the segment. The cirrus, which was protruded in many segments, is armed with extremely minute spines.

Female genital organs. The ovary is rather small, granular in appearance, and situated directly behind the vesicula seminalis. The vitelline gland is small, globular, and placed immediately posterior to the ovary. The vagina is so minute that it can be seen only with great difficulty. Its true course was not made out in our total mounts. The uterus is a typical sac, filling the entire segment. The vesicula seminalis remains a prominent structure even in gravid segments.

Remarks. Von Linstow (1906) briefly described this worm from the painted snipe, Rostratula capensis, from Ceylon. We thought it desirable to add the above details to his description.

Hymenolepis stylosa (Rudolphi, 1810) Volz, 1899

One complete worm, with a scolex, and six fragments from the intestine of Balopogon indicator indicator; Thysville.

The complete worm measures 4 cm. in length and has a maximum breadth of 1 mm. As usual in species of this genus, the segments are all broader than long. The genital pores are unilateral, and are situated in the anterior half of the lateral margin of the segment.

The rostellum bears 10 long slender hooks, each measuring 28μ in length and having the shape typical of this species.

The longitudinal excretory vessels are exceptionally well developed and prominent.

Of the three testes, two are aporal, situated one behind the other. The uterus is an irregular sac full of eggs, each of which measures 30μ in diameter.

The species has previously been recorded from Oriolus galbula in Africa.

Hymenolepis unilateralis (Rudolphi, 1819) Fuhrmann, 1906

Synonym: Hymenolepis ardeae Fuhrmann, 1906

Six fragments, two of which bear a scolex, from the intestine of *Butorides* striatus atricapillus; Kwango.

The largest fragment measures 9 cm. in length and 3 mm. in breadth. The genital pores are unilateral.

On one scolex the rostellum was evaginated to the extreme limit and the hooks had fallen off. In the second scolex the rostellum was strongly retracted and bore 10 hooks of the shape typical of this species, each hook measuring about 35μ .

The three testes are situated in line, on the pore side of the ovary. The cirrus pouch is small, not extending to the lateral excretory vessels. The cirrus

is thickly armed with rather large spines. In two lengths of strobila, each measuring 1 cm., the cirrus was protruded in every segment. In the anterior segments the cirrus was thickly matted with spines, but in the posterior ones the spines were entirely absent, indicating that they are of a deciduous nature.

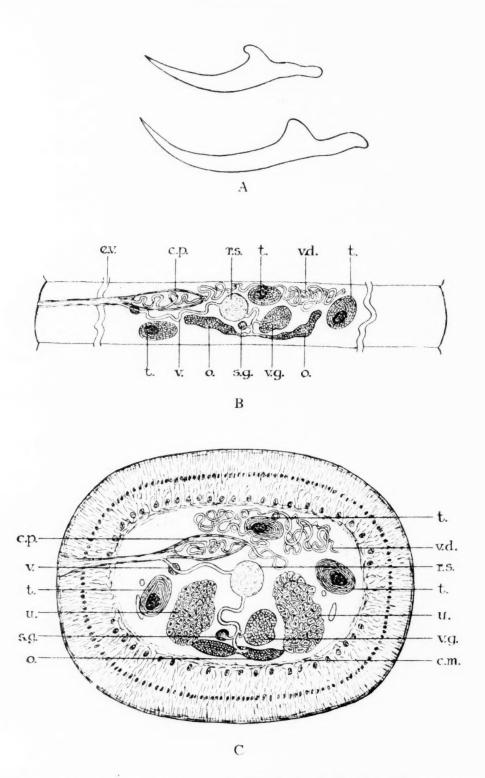


Fig. 4. Hymenolepis varicanthos sp. nov. A.—Hooks (\times 300). B.—Mature segment (\times 200). C.—Transverse section (\times 100).

The uterus is sac-like, and extends lateral to the excretory vessels.

Fuhrmann (1906) recorded this species from *Butorides virescens* in Central America. It has also been recorded from *Butorides striata*.

For a discussion of the synonomy of this species the reader is referred to Ransom (1909, p. 72).

? Hymenolepis fringillarum (Rudolphi, 1809) Fuhrmann, 1906

One specimen of what we believe to be this species was obtained from the intestine of *Dicrurus modestus coracinus*; Thysville.

The specimen was not well preserved, and it was impossible to make out details of the internal anatomy. All the segments are broader than long, and the genital pores are unilateral. The uterus is a transverse lobular sac.

The head is armed with 10 hooks, each one measuring 27μ and having the shape of the hook figured for this species by Fuhrmann (1906). It is for this reason that we think it probable that the species is *fringillarum*.

Hymenolepis varicanthos sp. nov.

About 10 specimens from the intestine of Ibis ibis; Kwango.

The worms measure about 1 cm. in length, and have a maximum breadth of about 1 mm. All the segments are extremely shallow, but they suddenly become almost square when gravid. The genital pores are unilateral.

The rostellum is well developed, and is armed with 20 hooks arranged in a double row, long hooks alternating with short. They have the shapes shown in fig. 4, A. These hooks vary in size on different rostella. The short ones vary from 74μ to 110μ , and the long ones from 130μ to 150μ .

The muscular system consists of a single outer layer of about 100 small bundles and an inner muscular layer of about 50 much larger bundles. The circular muscles are not well developed, and, as usual, lie internal to the inner layer of longitudinal muscles (fig. 4, C).

In some specimens the excretory vessels are situated at the junction of the middle and lateral thirds of the breadth of the segment; the whole of the genital organs in mature segments being in the middle third.

There are three testes, situated almost in line, but having a somewhat variable relative position. The cirrus pouch extends more than a third of the breadth of the segment. The cirrus is armed with minute spines.

The ovary is a bilobed organ, lying posteriorly in the centre of the segment. The vagina dilates into a globular receptaculum seminis just in front of the ovary. The vitelline gland is large and conspicuous, lying close to the ovary (fig. 4, B, C). The uterus is a transverse lobed sac completely filling the gravid segment.

Diagnosis. As far as we are aware, the only species of Hymenolepis at present recorded from Phoenicopteriformes are the following:

	N	o. of hooks	Size of hooks
H. flamingo	 	8	$57-62\mu$
H. megalorchis	 	8	90μ
H. caroli	 	8	130μ
H. liguloides	 	8	130μ
H. fanatica	 	? 6	$51-55\mu$

It is obvious that our species differs widely from all the above.

Of all other species of *Hymenolepis* which possess a rostellum armed with about 20 hooks, namely *H. coronula*, *H. simplex* and *H. micrancristrota*, the hooks are small and never exceed 20μ in length.

H. tetracis Cholodkovsky, 1906, has been recorded from Otis tetrax. In this species the rostellum is well developed and armed with from 16 to 20 hooks, each measuring from 92μ to 102μ in length. The hook is quite different in shape from that in our species, and, in addition, in H. tetracis the testes are in a straight line.

We are aware that our species closely resembles Oligorchis hierticos Johni, 1934. We were unable to find more than three testes. Johni's species was from Milvus govinda (Accipitriformes) from India, while ours is from Ibis ibis (Phoenicopteriformes) from the Belgian Congo. Since the rostellar hooks vary in size, we have named our species H. varicanthos.

Hymenolepis sp.

Several specimens without heads from the intestine of *Lophuromys* sp.; Kwango. In the absence of a head we found it impossible to identify the species. The mature segments contained three testes, however, and hence we refer the worm to the genus *Hymenolepis*.

Hymenolepis sp.

One specimen without a scolex from the intestine of Vinago calva calva; Kwango.

The strobila measured about 4 cm. in length by about 300μ in breadth. The genital pores are unilateral, and each segment contained three testes. It is not possible to identify the parasite further.

Hymenolepis sp.

A few specimens of an undetermined species of *Hymenolepis* were obtained from the intestine of *Melanobucco minor intercedens*; Kwango.

Two portions of strobila each carried a scolex, but in neither case was the scolex armed with hooks. We assume that they had been lost. The mature strobila were not well preserved, but we were able to ascertain that the genital pores were unilateral and that there were three testes in each segment.

Apparently species of *Hymenolepis* have not hitherto been recorded from Piciformes.

Genus II. Echinocotyle R. Blanchard, 1891

Echinocotyle rosseteri R. Blanchard, 1891

Eight complete worms and several fragments from the intestine of Spermaspiza haematina pustulata; Thysville.

The maximum length is from 2.5 cm. to 3 cm. and the breadth 1 mm. The rostellum is armed with 10 hooks, 40μ in length. The blade is somewhat shorter than the shaft, the ventral root being only slightly developed. The suckers are oval and armed round the margins with from 3 to 7 rows of rose-thorn-shaped hooks, each 5μ to 7μ in length; there are a few hooks at the centre of the sucker.

The genital pores are unilateral, and situated in the anterior part of the segment. The cirrus sac extends more than half-way across the segment. The testes are three in number, placed almost in a straight line between the lateral excretory vessels. The uterus is a transverse lobulated sac, and in a large number of segments it was filled with eggs.

Diagnosis. This species agrees, except in size, with E. rosseteri R. Blanchard, 1891, and differs from other known species of this genus, amongst other things, in the size of the hooks. Blanchard's specimens were obviously immature, and this probably accounts for the small size (3 mm.) given by Blanchard for the worm. Our specimens exhibited many gravid segments full of eggs.

Apparently this species has hitherto been found only in Anseriformes, although *E. tenuis* Clerc, 1906, *E. nitidulans* (Krabbe, 1882) Fuhrmann, 1906, *E. uralensis* Clerc, 1902, and *E. nitida* (Krabbe, 1869) Clerc, 1902, have been recorded from Charadriiformes.

We are therefore recording E. rosseteri for the first time from a passerine bird.

Genus III. Haploparaxis Clerc, 1903

Haploparaxis crassirostris (Krabbe, 1809) Clerc, 1903

Two specimens from the intestine of Galachrysia nuchalis nuchalis; Kwango.

The worms measure about 12 mm. in length and 1.5 mm. in breadth. Nearly all the segments are much broader than long. The genital pores are unilateral. The head is armed with 10 hooks, each measuring 37μ and having the shape characteristic of the species.

There is a single testes in each segment, situated on the pore side, close to the ovary.

The uterus is a lobulated sac lying transversely across the segment.

Neither of our worms was fully gravid. As far as we are aware, this is the first record of this species in *Galachrysia*.

Genus IV. Oligorchis Fuhrmann, 1906

Oligorchis kwangensis sp. nov.

Numerous specimens from the intestine of *Galachrysia nuchalis nuchalis*; Kwango. None of them was fully gravid. They measure about 1 cm. in length, and have a maximum breadth of 1.5 mm. The worms are lanceolate in shape, and all the segments are very much broader than long. The genital pores are unilateral.

Head. This measures 115μ in length and 160μ in breadth. There is a long rostellum armed with 10 minute hooks, each 12μ in length, and having the shape shown in fig. 5, A.

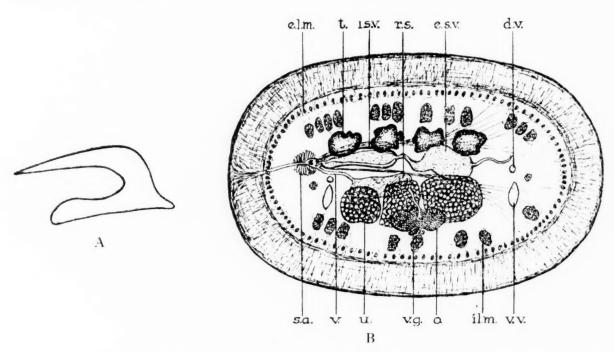


Fig. 5. Oligorchis kwangensis sp. nov. A.—Hook (2,900). B.—Transverse section (< 80).

Muscular system. The longitudinal muscles are in two layers. The outer layer, of about 70 bundles, completely surrounds the worm and is much smaller than the inner layer, which consists of about 12 bundles dorsally and 12 bundles ventrally. The circular muscles are very feebly developed, and could hardly be distinguished.

Excretory system. The excretory vessels are situated about one-fifth of the breadth of the worm from each lateral margin. They are both very small, the ventral vessel being slightly larger than the dorsal.

Male genital organs. There are from 4 to 7 testes, placed posteriorly and in line. The cirrus pouch is very large, extending half way across the segment; it is occupied almost entirely by an internal seminal vesicle. Attached to the poral extremity of the cirrus sac are two powerful muscles, one dorsal and the other ventral. They extend to the aporal margin of the medulla, diverging

as they go. There is a well-developed sacculus accessorius. The seminal vesicle when fully developed is a large and conspicuous sac.

Female genital organs. The ovary is bilobed and situated posterior to the testes. The vagina is rather short, and opens into a prominent receptaculum seminis, lying dorsal to the ovary. The vitelline gland is a minute globular mass, placed immediately behind the ovary (fig. 5, B).

The uterus is a transverse lobulated sac; it contained immature eggs

only.

Up to the present only seven species of Oligorchis have been recorded:

1. O. delachauvi Fuhrmann, 1909. From Pelicaniformes. This species has four testes. The head was not described.

2. O. strangulatus Fuhrmann, 1906. From Accipitriformes. This species has four testes only. There are from 14 to 16 hooks, each measuring 34μ .

3. O. yorkei (Kotlán, 1923). From Galliformes. In this species the rostellum has 50–52 hooks in a double row, the anterior ones measuring 135μ and the posterior ones $148–151\mu$. There are four testes.

4. O. longivaginosus Mayhew, 1925. From Pelicaniformes. The head is armed with a single crown of about 20 large hooks, each measuring from 88μ to 92μ . 'The testes are usually four in number' (Mayhew, 1925).

5 Ω hiertices Lohri 1934 From Accipitriformes The rostelly

5. O. hierticos Johri, 1934. From Accipitriformes. The rostellum bears from 16 to 18 hooks. Apparently these are placed in two rows, those in one row measuring from 83μ to 100μ , and those in the other measuring from 113μ to 190μ in length.

6. O. toxometra Joyeux and Baer, 1928. From Charadriiformes. In this species there is a single crown of 10 hooks, each of which measures from 37μ to 40μ in length. There are four slightly lobed testes.

7. O. paucitesticulatus Fuhrmann, 1913. From Charadriiformes. This species has 10 hooks, each measuring $16-18\mu$. Fuhrmann states that there may be from 7 to 11 testes.

In 1934 Johri erected a new genus, *Pseudoligorchis*, to accommodate a new species (*P. magnireceptaculata*) from a bat. He suggested that Fuhrmann's species *O. paucitesticulatus* should be placed in his new genus, on account of the fact that in this species there are from 7 to 11 testes.

It is perhaps correct to say that the essential characters of the genus Oligorchis are (1) that they are found only in birds; (2) that there is, apparently, always a double layer of longitudinal muscles; (3) an external and internal seminal vesicle are present; (4) they possess four testes.

In both O. paucitesticulatus and O. kwangensis, the new species described above, the number of testes is more than four. Johri's new species was from a bat; O. paucitesticulatus and O. kwangensis are both from birds (Charadriiformes). It is not probable that species of one genus would be found in both birds and bats. It appears to us that it would be better to accept Fuhrmann's emendation of the generic characters of Oligorchis, rather than to place together

in a somewhat artificial genus tapeworms from such widely different hosts as birds and mammals.

In Johri's new genus it is not known with certainty whether the rostellum is armed or not, but it is believed to be unarmed, as Johri was unable to find any hooks either in the living worms or in the fixed preparations.

We therefore retain our new species in the genus *Oligorchis* as emended by Fuhrmann.

It will be noted that our worm differs from all other species hitherto described, but is most closely related to O. toxometra Joyeux and Baer, from which it differs in the size (but not in the shape) of the hooks, and in the number of testes. It is also interesting to note that O. toxometra was obtained from Gallinago sp. in French Guinea.

(To be continued)

A FINAL NOTE ON A STRAIN OF TRYPANOSOMA GAMBIENSE TRANSMITTED THROUGH MONKEYS BY GLOSSINA MORSITANS

BY

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In the preceding note (Corson, 1938) the duration of life of monkeys was given in a table which also showed the results of examination of the cerebrospinal fluid of those monkeys which were chloroformed when in the last stages of the disease or for other reasons. Some miscalculations were made in the table: monkey 115 had lived for 238 days, instead of 537, as stated; and monkey 110 had lived for 202 days, and not for 501. It must be added that monkeys 54 and 76 were exceptional, in that trypanosomes had not been seen in the blood for many months, but examinations had been infrequent. The state of the cerebrospinal fluid of monkey 54 would suggest that it had recovered, but a comparison with monkey 76 makes it doubtful; the latter monkey seemed to have recovered, as it was strong, active and well-nourished, and daily examinations of stained thick blood-films from October 24th to 31st, from November 16th to 23rd, and from November 28th to December 7th, 1938, showed no trypanosomes. On December 12th, however, one trypanosome was found in 200 fields, two in 200 fields on December 17th, one in 200 fields on December 18th, and none on December 19th. Daily examinations of thick films were again made from January 4th to 11th, 1939, and trypanosomes were only seen on the last day, when the film showed two in 200 fields. The monkey was chloroformed on January 17th, and cerebrospinal fluid was withdrawn by suboccipital puncture; no trypanosomes were seen, and there were 35 leucocytes in a cubic millimetre. A thick blood-film showed one trypanosome in 200 fields.

Among the monkeys of the series of animals which were infected by inoculation, no. 78 seemed to have recovered; daily examinations of thick blood-films in October, November, December, 1938, and January, 1939, showed no trypanosomes, and the monkey was apparently in good health. It was chloro-formed on January 17th and the cerebrospinal fluid was examined; no trypanosomes were seen, but there were about 1,000 leucocytes per cubic millimetre. I do not think that it can be judged to have recovered from its infection. The other remaining monkeys of the series infected by inoculation were nos. 114, 101, 112 and 113. Monkey 114 was chloroformed on the 299th day after inoculation, and its cerebrospinal fluid showed one trypanosome and 800 leucocytes in a cubic millimetre. Monkey 101 was chloroformed on the 417th day after inoculation, and its cerebrospinal fluid showed no trypanosomes but over 5,000

leucocytes per cubic millimetre; both these monkeys were weak and ill when chloroformed. Monkey 112 was chloroformed while in apparently good health on November 19th, 1938, the 404th day after inoculation; trypanosomes had not been seen in its blood for many months; the cerebrospinal fluid showed no trypanosomes, but there were 259 leucocytes in a cubic millimetre; the total protein, estimated in 2 c.cm. of fluid in a Sicard and Cantaloube tube, was 0.022 per cent.; it is doubtful whether the monkey had recovered. Monkey 113 was also apparently in good health when chloroformed on November 19th, 1938, the 384th day after inoculation; no trypanosomes had been seen in its blood for many months, and, as with monkey 112, daily examinations had been made

TABLE

emarks	Cerebrospinal Length fluid of life			Died on	Blood last bied on		
emarks	Ken	White cells	Living tryps.	in days	Died on	on	no.
ed in poor hea	Chloroformed	640	50	1,174	15.1.39	15,1,39	32
good .	**	170	0	1,138	16.1.39	19,11,38	33
11	.,	370	()	1,127	16.1.39	30,11,38	3.5
12	**	250	1	1,056	16.1.39	16, 1.39	40
,, .,	**	350	7	1,047	16.1.39	16.1.39	43
,, ,,	**	400	2	1,007	16.1.39	16.1.39	45
		60	0	987	16, 1, 39	16,1,39	48
022 per cent.	$\begin{cases} \\ \text{protein } 0.02 \end{cases}$	7	()	885	19.11.38	10.1.37	54
	Chloroformed	150	:3	777	17.1.39	17.1.39	7.5
**	**	35	()	771	17.1.39	17.1.39	76
,, ,,		25	()	439	17.1.39	17.1.39	115
.022 per cent.	$\begin{cases} & \text{"}\\ \text{protein } 0.02 \end{cases}$	44	()	344	19,11,38	13,3,38	110
ed in good hea		55	()	289	17.1.39	17,1,39	133

for a week in October and from November 16th to 19th. Its cerebrospinal fluid, which was slightly contaminated with blood, showed no trypanosomes and 56 leucocytes in a cubic millimetre, of which 16 should be deducted to allow for the contamination with blood; an estimate of protein could, of course, not be made.

Chronic infections of monkeys with *T. gambiense* were observed by Bruce et al. (1915) and by Duke (1931); Van Hoof (1934) recorded spontaneous recovery of monkeys, *Cercopithecus* sp., and referred also to cases of spontaneous recovery in man. The interest of such infections in susceptible monkeys is, of course, that they suggest that similar chronic infections, possibly undiagnosed,

may occur in man; it may also be thought that such infections have a bearing on the use of volunteers in experimental work with human trypanosomes, indicating the advisability of treating all volunteers, whether showing infection or not, with germanin, after, in the latter case, an interval within the limit of safety.

About the end of 1938 I decided to end the experiment by chloroforming the remaining monkeys and examining the cerebrospinal fluid microscopically. The results are shown in the table.

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A SECOND NOTE ON SUCCESSFUL TRANSMISSION OF ORIENTAL SORE BY THE BITES OF STOMOXYS CALCITRANS

BY

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In a previous paper (Berberian, 1938) we reported two successful transmissions of oriental sore by the bites of *Stomoxys calcitrans*, and stated that a fuller report of our experiments would be published at a later date. The long incubation-period of the disease, and the slow development of the lesions, prolong the course of these experiments, and, inasmuch as the results of these experiments are awaited with great interest, we decided to report two more successful transmissions and to describe in detail the development of the two previous sores reported in 1938.

The course of development of the sores previously reported. One of these sores appeared on December 23rd, 1937, and the second on January 22nd, 1938, after an incubation-period of five months in the case of the first sore, and six months in the case of the second. On March 15th, 1938, the lesions were photographed. At that time the first sore was 4 mm. in diameter, and the second 2 mm. in diameter (fig. 1). The papules were so close to each other that as they



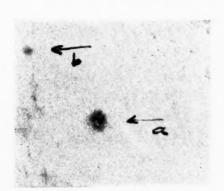


Fig. 1

Fig. 2

Fig. 3

Experimental oriental sores transmitted by the bites of Stomoxys calcitrans

grew larger they fused together, and after April 15th, 1938, some sero-sanguincous material containing *Leishmania tropica* was obtained from the lesion and was successfully cultured on NNN medium. The culture was later used for the production of experimental sores on volunteers. In July, 1938, this lesion was used for the transmission experiments described below, and at that time it was about 12 mm. in diameter. In October, 1938, the sore ulcerated and began to ooze (fig. 2). Since December, 1938, it has been regressing slowly. A smear prepared on January 20th, 1939, showed no Leishman-Donovan bodies. Four volunteers were used in this first series of transmission experiments with S. calcitrans, and one of the four volunteers developed two sores.

Two more successful transmissions by the bites of Stomoxys calcitrans. On July 26th, 1938, three stable-flies were fed on the lesion of volunteer no. 2. described above, and were immediately transferred to the anterior aspect of the left thigh of volunteer no. 6. These flies bit the sore ten times and the thigh of the volunteer five times. On December 14th, 1938, approximately five months after the bites, the volunteer noticed for the first time a pin-point violaceous papule at the site of the bites. On December 29th, 1938, two weeks later, one more pin-point papule was noted at a distance of about 1 cm. from the first papule. These two lesions were photographed on January 17th, 1939. At the time of the photographing, the first lesion was about 3 mm. in diameter and the second about 2 mm. (fig. 3). Examination of smears prepared from the lesions showed numerous Leishman-Donovan bodies. Three volunteers were used in this second series of transmission experiments with S. calcitrans, and one of them developed two sores. Thus two more successful direct mechanical transmissions of oriental sore by the bites of S. calcitrans were obtained, so far making four successful experimental transmissions.

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A FURTHER NOTE ON SOME AFRICAN VOLUNTEERS IN EXPERIMENTAL WORK WITH TRYPANOSOMA RHODESIENSE

BY

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(Received for publication March 1st, 1939)

In a recent paper (Corson, 1938) I recorded a somewhat high incidence of various illnesses in Africans who had volunteered for experimental infection with *Trypanosoma rhodesiense*. These illnesses occurred during or after treatment with germanin, both in patients who had become infected and in others who had resisted. I concluded that there was no reason to attribute these complications, except albuminuria, to the action of germanin or to infection with *T. rhodesiense*, and suggested that such illnesses were not exceptional in the local population to which the volunteers belonged.

That paper was written about the middle of October, 1938, and from September, 1938, to the present time (February, 1939, when my opportunities for further observation are about to cease) I have been able to see nearly all the volunteers weekly.

Volunteer no. 39 had died, so that 42 remained; four of these, nos. 4, 22, 36 and 38, had moved from the district, and I therefore did not see them. Of the remaining 38, only three, nos. 27, 28 and 42, had anything more than very trivial disturbances of health: volunteer no. 27 had diarrhoea in October, and a short attack of acute bronchitis with fever for two days in November; volunteer no. 28 had diarrhoea and an undiagnosed fever of four days' duration in November; and volunteer no. 42 suffered from pain in his left foot, which began on December 12th and lasted till January 6th, and for which no local cause was found. He had received intravenous injections of 1 gm. doses of germanin on August 21st, 24th, 28th, September 10th and 18th, 1938. He was a strongly built, hardy man, and I do not doubt the correctness of his statement that the pain was severe at times. His case is interesting, because a few other volunteers had complained of similar pains during or soon after treatment, and one of them, no. 43, obviously suffered very severe pain. Lester (1936) reported mild peripheral neuritis after the administration of germanin or antrypol, and thought that the drug had caused it.

Excluding such infections as malaria, relapsing fever, schistosomiasis, filariasis, abscess and venereal disease, 13 of the volunteers (nos. 6, 9, 17, 21, 23, 27, 34, 35, 36, 37, 39, 40 and 43) were recorded in the previous paper as having had some disturbance of health during or soon after their course of treatment with germanin. The 38 volunteers who have been under close observation during the last few months have kept so well in health that I am

now doubtful whether germanin did not play some part in the production of those illnesses; whether or not that was so, the volunteers seem now completely to have recovered.

As it may possibly be useful for future reference, a table of the volunteers is given, which shows the periods within which they received their injections of germanin; most of the volunteers received four doses, and when fewer doses were given it was because of excessive albuminuria or intercurrent illness.

TABLE OF VOLUNTEERS

Name	No.	Date of fly-bite	Infected	No. of	Germanin in 1 gm. doses days after the first dose			
		(or inoculation)	resisted	doses	2nd	3rd	4th	5th
Machivia	1	21.3.37	I	4	3	7	14	
Masungwi	2	$\begin{array}{c} 22.5.37 \\ 3.6.37 \end{array}$	R	2	6			
Tuga	3	5,6,37	I	4	4	7	14	
Bundala Kasseko	4	5.8.37 17.8.37	Ŕ	4	5	12	23	
Sunzura	5	23.8.37	ı	4	4	8	27	
Sija Chambi	6	22.12.37	I	4	3	7	13	
Kabati	7	13.12.37	I	4	4	10	15	
Osmani	8	(inoculation) 30.12.37	R	3	2	6		
Nkununazi	9	(inoculation) 17.1.38	I	4	4	8	25	
Bundala Nyumbu	10	(inoculation) 3.2.38	R	4	4	8	15	
Mganga Sija	11	7.2.38	I	3	4	8		
Masaga	12	6,2,38	I	4	4	8	19	
Bundala Fukarra	13	17.2.38	I	4	3	7	12	
Sombi	14	18.2.38	I	4	4	8	15	
Nungu	15	23.2.38	1	4	4	7	15	
Ganai	16	23.2.38	I	4	4	9	14	
Mihambo Masesa	17	24.2.38	I	4	6	13	27	
Hamisi	18	24.2.38	I	4	4	8	14	
Mganga Tubari	19	27.2.38	I	4	5	8	14	
Tifundo	20	4.3.38	1	4	5	9	20	

TABLE OF VOLUNTEERS (Continued)

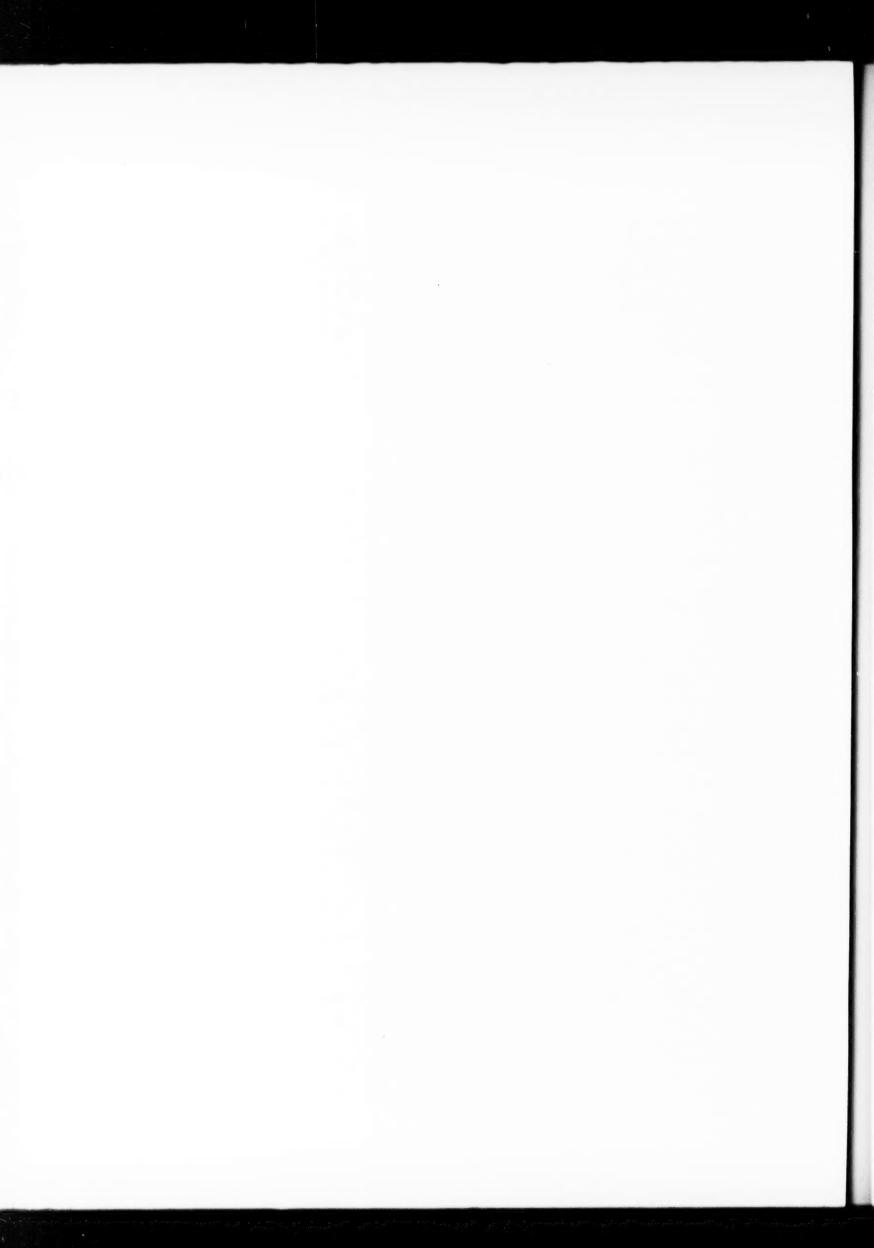
Name	No.	Date of fly-bite	Infected	No. of	Germanin in 1 gm. doses days after the first dose			
		(or inoculation)	resisted	doses	2nd	3rd	4th	5th
Mashimba	21	4.3.38 12,3.38	R	3	3	6		
Masere	22	24,3,38	I	4	4	8	16	
Masissa	23	15,3,38	R	4	3	7	21	
Dotu	24	15.3.38	I	4	4	8	14	
Ndegwa	25	17.3.38 30.3.38	R	4	3	7	21	
Manyesha	26	18,3,38	I	4	5	8	13	
Kajiga	27	20,3,38	R	4	3	24	30	
Kajala	28	21,3,38	I	4	4	8	16	
Mange	29	22,3,38 11,4,38	R	4	3	7	17	
Shimmo	30	23,3,38	I	4	5	8	21	
Mati	31	29,3,38	R	4	4	8	15	
Sija Maggi	32	16.4.38	I	3	3	7		
Angayu	33	27.4.38	1	4	3	10	16	
Mahona Maguja	34	24,4,38	I	3	4	8		
Kashinji	35	24,4,38	R	3	3	10		
Masunga	36	13,4,38	R	3	6	13		
Mihambo Doto	37	19.4.38	R	2	4			
Lajabu	38	14,4,38	I	4	5	8	17	
Mihambo Kukuwa	39	22,4,38	I	4	5	11	19	
Fukarra Kisabu	40	$\begin{array}{c} 12.7.38 \\ 27.7.38 \end{array}$	R	5	4	18	29	39
Mishari	41	$\frac{29.7.38}{13.8.38}$	R	5	3	8	23	37
Mahona Gayni	42	11,8,38	I	5	3	7	20	28
Ndugu	43	15,9,38	I	4	4	7	14	

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THE SUSCEPTIBILITY OF THE MOSQUITO AËDES TRISERIATUS TO THE VIRUS OF YELLOW FEVER UNDER EXPERIMENTAL CONDITIONS

BY

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The mosquito Aëdes triseriatus (Say, 1823) is neoarctic in its distribution and has been described as occurring from Maine to Florida and westward to Montana and Texas, a region which lies outside the endemic zones of yellow fever. In his report of the habits and life-history of the mosquitoes occurring in New Jersey, Smith (1904) gives a comprehensive description of this species under the designation *Culex triseriatus*. The following paragraph from Smith's report (page 277), concerning the larval stage of the species *triseriatus*, is of especial significance:

'This larva, in appearance, is nearer like that of *Stegomyia fasciata*, the yellow fever mosquito, than any other of those that occur in New Jersey. Whether this indicates a closer relationship than the adults seem to show and, whether at a pinch, *triseriatus* might do the work of *fasciata* in transmitting the yellow fever, are interesting speculations.'

Independently of this suggestion, it occurred to one of us (Baker) to test this mosquito for its susceptibility to the virus of yellow fever.

EXPERIMENTS ON TRANSMISSION

Larvae of A. triseriatus were collected from tree-holes in the region of Ithaca, New York, and a colony was established quite successfully. Under circumstances designed to imitate conditions occurring in nature, this species propagated readily in a greenhouse type of insectarium at Cornell University (Baker, 1937, 1938). The experiments in transmission were conducted at the

Harvard Medical School, using a technique which has proved quite satisfactory with other mosquitoes (Sellards, 1932).

Observations on Monkeys. In the autumn of 1935, about 35 triseriatus mosquitoes (lot no. 1) fed vigorously and promptly on a monkey (A) which was dying of yellow fever. These mosquitoes were kept during their incubation-period at a temperature of 28° C. and a relative humidity of not less than 70 per cent. At the end of 17 days, only 7 remained alive, and 6 of these fed on a rhesus monkey, Macaca mulatta (no. 1). This animal remained in excellent condition, the temperature being within normal limits, except for a slight febrile reaction (104·1° F.) which occurred on the sixth day. In order to determine the nature of this reaction, the susceptibility of the monkey to yellow fever was to have been tested one month after the mosquitoes had fed. There happened to be a slight delay in these arrangements, however, no immunity-test was given, and the monkey died of yellow fever 36 days after being bitten by mosquitoes. Thus, if virus had been injected as planned on the 30th day of the experiment, the death of the monkey 6 days later from yellow fever would have yielded a conclusion entirely erroneous in every respect.

It became necessary to discontinue the colony of mosquitoes on account of the departure of Dr. Baker from Cornell. In the following summer, occasional attempts were made to rear triseriatus mosquitoes in Boston, using a plan only slightly more complicated than the technique which is quite adequate for A. aegypti. Larvae of triseriatus were kindly supplied by Professor T. J. Headlee and Mr. R. L. Vannote, of the New Jersey Agricultural Experiment Station; and a few triseriatus larvae were obtained from the peninsula of Florida. The attempts to establish a colony with this simplified technique were unsuccessful. On the return of Dr. Baker to Cornell University, larvae of triseriatus were collected in March, 1938, and an adequate supply of mosquitoes soon became available for experimentation. After taking an infective blood meal from a monkey sick with yellow fever, some of the mosquitoes were incubated at 28° C., with a relative humidity of about 70 per cent. (lots 2 and 3), whereas others (lot 4) were kept at 37° C. with a humidity approaching saturation. Lot 2 (about 40 mosquitoes) received its infective feeding from a monkey (B) during its second day of fever. A titration was made to determine the infectivity of the monkey's blood at this time. Serum in ten-fold dilutions was injected in quantities of about 1/60 c.cm. into the brains of white Swiss mice; at a dilution of 1 to 100,000 all the mice died, and at 1 to 1,000,000 half of the mice died of Lot 3 and lot 4, numbering about 45 mosquitoes each, fed simultaneously on a dying monkey (C). A titration was made of the serum of this animal, and a dilution of 1 to 1,000,000 killed 3 of 4 mice upon intracerebral injection. After an appropriate interval, these three lots of mosquitoes fed on four rhesus monkeys. One monkey (no. 2) was bitten by 5 mosquitoes (lot 2) 14 days after their infective feeding. Another monkey (no. 3) was bitten by 5 mosquitoes (lot 3) 15 days after their infective feeding, and on the following

day this monkey was bitten by 3 mosquitoes of this lot. Both these monkeys remained well and developed no febrile reaction. The blood of these animals failed to infect mice on intracerebral injection. Moreover, specimens of blood which were taken at the end of 6 weeks failed to protect mice against yellow fever, using a suspension of the brain of a mouse infected with neurotropic virus. This was centrifugalized in order to remove the tissue, and the supernatant fluid was mixed with the serum to be tested. 'The mixture was injected intraperitoneally in mice according to a technique already described (Sellards and Bennett, 1937). The 45 mosquitoes (lot 4) which were kept at body-temperature survived very well indeed, and at the end of one week about 39 were alive and appeared to be in good condition. They were removed to ordinary roomtemperature and were given an opportunity to feed on a normal monkey (no. 4), but in the course of two hours only one took any blood. This lot of mosquitoes was transferred to 28° C., and on the following day 7 took a full feeding on this monkey (no. 4). After 48 hours at room-temperature (28° C.), these mosquitoes (lot 4) were allowed to bite another normal monkey (no. 5); 7 fed vigorously, and on the following day 3 mosquitoes took blood. Neither of these two monkeys developed any febrile reaction, but both died of yellow fever 10 and 13 days after being bitten. At autopsy neither monkey showed jaundice nor gastric haemorrhages, but in both the spleen was tense, the liver was fatty, and the sections of the liver showed an unmistakable picture of yellow fever.

Intracerebral Injection of Mice. At the conclusion of the feeding experiments on monkeys, a few mosquitoes from lots 2, 3 and 4 were fixed in Zenker and stained with Giemsa for histological study, but the sections showed nothing of special interest. Other mosquitoes were tested for virus by intracerebral injections in white Swiss mice. Each mosquito was ground in an agate mortar with about 2 c.cm. of physiological saline containing 10 per cent. of normal horse serum. This suspension was centrifugalized for 10–15 minutes at moderate The supernatant fluid was injected intracerebrally in three or four mice and a drop was inoculated on an agar slant. Ten mosquitoes were tested in this manner. In about half of these the supernatant fluid showed a growth of saprophytic bacteria, but the mice inoculated with the corresponding fluid showed no evidence of any secondary infection at any time. Sixteen of 32 mice died of yellow fever, the virus being recovered from 6 of these 10 mosquitoes. The fatal infections in mice were characterized by the appearance of symptoms of paralysis after the usual period of incubation. Histologically, sections of the brain showed evidence of yellow fever. The incubation of brain tissue on agar slants failed to show any saprophytic contaminants. There was a lack of correlation between the effects of the injections in mice and the results of the feeding experiments on monkeys. Virus was demonstrated in each of the three lots of mosquitoes (nos. 2, 3 and 4) by the injection of mice, whereas only lot 4 produced infection when allowed to feed on monkeys. The details of these data are shown in the following table.

TABLE

Infectivity of A. triseriatus as tested by feeding experiments on monkeys and by the intracerebral injection of white mice with suspensions of mosquitoes

Lot no.		Individual mosquitoes	Inter afte infec feed	er tive	No. of mice injected	No. mice of of yel feve	lead low	Results of feeding experiments on M. mulatta
1	28	No	t tested	l on	mice		{	One monkey bitten and died of yellow fever after 36 days
2	1.0	A	17 da	VS.	4	0)	One monkey bitten and
9	.,	В	17		4	4	Ì	remained well
3	,,	C	16	1.0	3	1	Í	
:3	,,	D	16	. 1	3	0	į	One monkey bitten and
3		E	16		3	3	7	remained well
3	**	\mathbf{F}	16		3	2	j	
4	37	G	13		3	0)	T
4		H	13	7 *	3	0	1	Two monkeys bitten and
4		I	13	* *	3	3	>	died of yellow fever 10
4	• 1	J	13		3	3)	and 12 days later
l'otals 3*		10			32	16		

^{*}Of the lots which were tested on mice.

Preliminary observations were made with the object of using mice in the absence of the usual laboratory facilities for investigating the question of sylvatic yellow fever. Suspensions of several arthropods were prepared in serumsaline, and injections were made intracerebrally in mice with no centrifugalization. Mosquitoes (Aëdes and Culex), the common water-cockroach (Blattella germanica) and the bedbug (Cimex lectularius) were tested in this manner. These suspensions were moderately rich in saprophytic bacteria, but the mice, 57 in all, withstood the injections well. Only 4 died and the majority remained free from symptoms, though some showed obvious cerebral involvement for one or two days and then recovered completely. Even under the conditions of working in the field, any infection due to yellow fever could accurately be diagnosed by simple methods.

DISCUSSION

The mosquito Aëdes triseriatus in its susceptibility to the virus of yellow fever occupies a position intermediate between species such as A. aegypti, which are extremely susceptible in contrast to those which are altogether refractory. Three of five monkeys developed fatal yellow fever after being bitten by infected triseriatus mosquitoes, but without any definite febrile reaction and without jaundice. The minimum course of the disease was 10 days, instead of the usual period of 4 or 5 days following infection by A. aegypti. One monkey died

36 days after being bitten by mosquitoes. This course of the disease is the longest that has occurred in our experience, and it exceeds the lengthening of the incubation-period resulting from injection by ordinary routes of minute amounts of virus. The atypical features observed under experimental conditions are reminiscent of some of the rare complications seen in man, such as an unexpectedly fatal termination in a patient who, presumably, has been convalescent for several weeks.

There is no correlation between the distribution of A. triseriatus and the epidemiology of yellow fever, and epidemiological observations would have failed to suggest that this mosquito might transmit yellow fever. In considering whether the virus could be maintained experimentally by serial passage in A. triseriatus, it is well to remember that even with A. aegypti some authorities, notably Davis and Shannon (1929), have reported many failures (50 per cent.) under experimental conditions, though at the same time more than 80 per cent. of infections resulted from the direct injection of blood. The palearctic mosquito A. geniculatus is widely distributed in Europe, and has been described in northern Africa and in Asia Minor. Roubaud and his collaborators (1937) noted that this species transmits vellow fever effectively under experimental conditions, the mosquitoes having been incubated after their infective feeding at 30° C. to 35° C. One of us (Sellards, 1930) noted that the incubation-time in aegypti was shortened to a few days when the mosquitoes were incubated at 37° C.

SUMMARY

Under experimental conditions, the mosquito Aëdes triseriatus transmitted vellow fever to the monkey Macaca mulatta (syn. Macacus rhesus). Four lots of mosquitoes were given infective feedings of highly virulent blood. After an appropriate incubation-period two of these lots, one of which had been kept at 37° C., produced a fatal infection when allowed to feed on monkeys. Mosquitoes of the other two lots failed to produce either infection or immunity in monkeys, although they harboured the virus. Encephalitis characteristic of vellow fever developed in white mice which were infected intracerebrally with ground suspensions of some of these mosquitoes.

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ON A COLLECTION OF CESTODA FROM THE BELGIAN CONGO

(Continued from p. 90)

BY

T. SOUTHWELL

AND

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(Department of Parasitology, Liverpool School of Tropical Medicine)

(Received for publication February 1st, 1939)

FAMILY 4. HYMENOLEPIDIDAE RAILLIET AND HENRY, 1909 (con.) SUBFAMILY II. DILEPININAE FUHRMANN, 1907 Genus I. Dilepis Weinland, 1858

Dilepis irregularis sp. nov.

Six specimens obtained from Rostratula benghalensis; Kwango. They were found along with large numbers of H. spinosa von Linstow, 1906. The worms measure from 2 to 3 cm. in length, and about 2 mm. in maximum breadth. In most of the specimens, two of which are gravid, all the segments are broader than long; but in portions of two worms, which have extended abnormally, some of the immature segments are longer than broad, even though the gravid ones in the same strobila are square, or broader than long.

The genital pores are unilateral, and are situated at the extreme anterior

margin of the segment.

Head. This measures about 200μ in length and 300μ in breadth. It is armed with a double row of hooks, of which there are 13 in each row. Each large hook measures 56μ in length, each small one 37μ . They have the shape shown in fig. 6, A.

Musculature. Owing to the fact that we had only a few worms, transverse sections were not made; but, in specimens mounted complete, the internal anatomy was partly obscured by the longitudinal muscles, which are strongly developed and consist of very large numbers of bundles.

Excretory system. This was not studied in detail, but in whole mounts a small vessel was conspicuous on each side, traversing the entire length of the

worm in broad wide curves.

Male genital organs. There are about 36 testes, situated posterior and lateral to the ovary. The cirrus pouch is large and conspicuous, running parallel and very close to the anterior margin of the segment; it narrows at its median extremity to a long, wide, and very convoluted vas deferens.

Ovary. This is an irregularly shaped organ, surrounded posteriorly and laterally by the testes and bounded anteriorly by the receptaculum seminis and vas deferens. The vagina is a rather wide tube, running posterior to the cirrus pouch; it dilates into a large seminal vesicle (fig. 6, B).

Uterus. This appears at first to be a series of oval bodies arranged across the segment, with their long axes directed antero-posteriorly. On closer examination, however, it is clear that in reality the uterus in its early development is a lobulated sac. When fully developed it completely fills the segment.

Diagnosis. We placed this worm in the genus *Dilepis* Weinland, 1858, on account of the fact that the head is armed with a double crown of hooks, the genital pores are unilateral, the testes are posterior and lateral to the ovary, and the uterus is a simple sac.

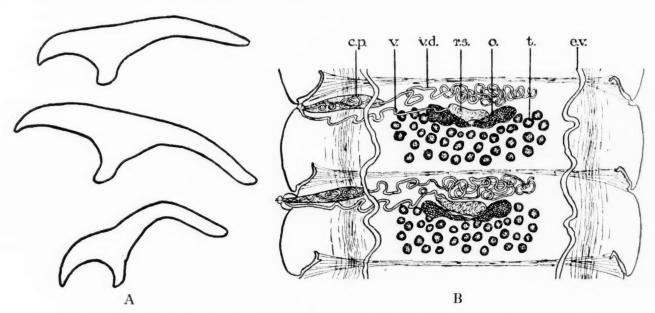


Fig. 6. Dilepis irregularis sp. nov. A.—Hooks (× 950). B.—Mature segments (× 80).

The appearance of the hooks suggested to us the possibility of the species belonging to the genus Anomotaenia Cohn, 1900, as many species of this genus have hooks identical in appearance with those of our worm. Furthermore, one of the characters of the genus Anomotaenia is the fact that the genital pores are situated near the anterior border of the segment, as they are in our species. But in the genus Anomotaenia the genital pores are irregularly alternate.

A large number of species of *Anomotaenia* have been recorded from Charadriiformes, although only about 10 species of *Dilepis*.

The hooks in our species somewhat resemble those of *Dilepis undula* (Schrank, 1788); but in the latter there are from 46 to 64 hooks, those in the anterior row measuring from 84μ to 105μ and those on the posterior row from 78μ to 80μ . Our parasite differs from all others hitherto described in the number and size of the hooks.

Genus II. Echinorhynchotaenia Fuhrmann, 1909

Echinorhynchotaenia tritesticulata Fuhrmann, 1909

Two complete worms and a few fragments from the intestine of *Anhinga* rufa rufa; Kwango.

The complete worms measured from 7 cm. to 8 cm. in length, the maximum breadth being 6 mm. and the thickness of the strobila 1.5 mm. The segments are extremely shallow. In the immature part of the strobila there are six segments to the mm., and three segments to the mm. in the gravid part of the worm. The genital pores are unilateral.

The head bears four suckers, each having a diameter of 370μ , and is marked by a deep rostellar sac, from which arises a conspicuous rostellum which is visible to the naked eye. Under magnification it appears to be a flattened, non-muscular,

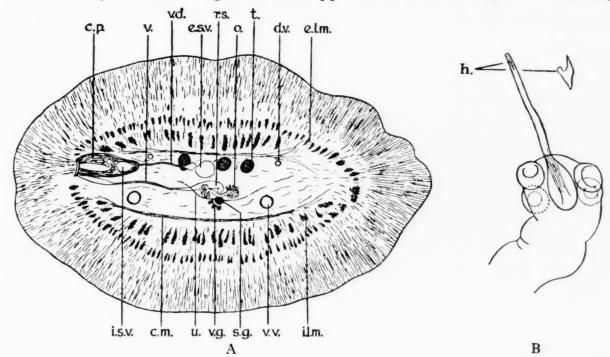


Fig. 7. Echinorhynchotaenia tritesticulata. A.—Transverse section (\times 26). B.—Head with rostellum (\times 800), and hook (\times 45).

rigid, chitinous-like rod, having a length of 560μ and a breadth of 40μ . The distal twentieth of the rostellum is armed, in the middle of the flat surface, with a large number of extremely minute hooks. We were unable to count them, as they were very irregularly crowded together, but we think that there must be about 40. Each hook had the shape shown in fig. 7, B, and measured 7.4μ in length.

The rostellum appeared so unusual—in fact, unique—that the rostellum on the second worm was also examined and found to be exactly similar to that described above. We are of the opinion that the whole of the rostellum was armed with hooks, though the second rostellum was exactly like the first. In Fuhrmann's specimens from the same host the rostellum was armed along its whole length, and retracted within the rostellar sac.

The excretory systems calls for no remark, except that the ventral vessel is larger than the dorsal vessel.

The muscular system is powerfully developed. The internal circular muscular fibres are conspicuous, demarking the cortex from the medulla. The longitudinal muscle fibres are in two layers, the internal one consisting of about 50 bundles, which are much larger than those of the outer layer, which consists of about 100 (fig. 7, A).

There are three testes situated dorsally, and in nearly all segments they lie in a straight line. The cirrus pouch extends to the dorsal excretory vessel. It contains a rather small, but nevertheless conspicuous, internal seminal vesicle. The cirrus is very large, powerfully armed, and entirely covered with innumerable minute spines, each one measuring about 5μ . The vas deferens proceeds almost as a straight tube to the middle of the segment dorsally, where it dilates into a rather peculiarly shaped seminal vesicle.

The ovary is situated ventrally and is bilobed, each lobe consisting of acini arranged fan-wise. The vitelline gland is small, diffuse, and placed ventral and posterior to the ovary. The vagina is a very delicate tube, which proceeds from the genital pore, running dorsal to the cirrus pouch. No receptaculum seminis was observed on the vagina in our sections, which were made from slightly immature segments (fig. 7, A).

The uterus is a transverse lobulated sac. In neither of our specimens were the eggs fully developed.

Subfamily III. Paruterininae Ransom, 1909 Genus I. Anonchotaenia Cohn, 1900

Anonchotaenia bobica Clerc, 1903

Several specimens from the intestine of Tshagra senegala rufofusca; Thysville.

The worms measure about 1 cm. in length and have a maximum breadth of 1 mm. None of them are gravid. The segments are extremely shallow, having a length of less than 100μ . The genital pores are irregularly alternate, and the head is unarmed.

The partly gravid segments exactly resemble the figure given of this species by Clerc (1903).

As far as we are aware, the species has hitherto been recorded only from the Ural mountains, and has been found in Sitta uralensis, Parus major and Chrysomitris spinus.

Genus II. Biuterina Fuhrmann, 1902

Biuterina meropina (Krabbe, 1869) var. macrancristrota Fuhrmann, 1908

Ten specimens from the intestine of Merops nubicoides from Kwango, and some specimens from Dryoscopus angolensis angolensis.

The worms measure about 3 cm. in length and 1 mm. in breadth. The mature and gravid segments are bell-shaped. The genital pores are irregularly alternate and situated near the middle of the lateral margin.

Head. This bears two rows of hooks, about 20 in each row, those in the anterior row measuring 38μ and those in the posterior 26μ . They are identical in shape with the hooks of this species figured by Fuhrmann (1908b).

Male genital organs. There are 8 testes in each mature segment in our

specimens. The cirrus pouch is relatively long and stout.

Female genital organs. The ovary is very small and bilobed. Immediately posterior to it is the minute vitelline gland. The uterus arises as a double sac. The paruterine organ develops immediately in front of the uterus proper, as a stout fibrous organ eventually occupying almost half of the segment.

Diagnosis. Krabbe (1869) obtained a worm from Merops superciliosus in Africa, which he named Taenia meropina. Fuhrmann (1908b) referred the

species to his genus Biuterina Fuhrmann, 1902.

In the same paper (1908b) Fuhrmann described a new variety of B. meropina, which he named macrancristrota. He obtained it from the intestine of Merops apiaster Lin. and Melitophagus albifrons. The distribution is given as Central and South Europe, Central Asia, India, and North and West Africa. As noted above, our specimens were from Merops nubicoides in Kwango.

Biuterina cylindrica Fuhrmann, 1908

Two fragments, each with a head, of what we believe to be this species were obtained from the intestine of *Astimastillas falhensteini*; Thysville. The scolex was armed with 52 hooks arranged in a double row; each hook measured 22μ and had the exact form figured by Fuhrmann for this species (1908b).

It is, perhaps, unusual to make a diagnosis of a species on a scolex only, but the number, size, and especially the typical shape of the hooks, leave us

practically certain that the species is B. cylindrica.

Fuhrmann recorded this species from Tachyphonus cristatus and Tachyphonus metaleucus from Brazil. It may be noted that both Astimastillas and Tachyphonus are passerine birds.

Biuterina sp.

Two fragments, composed of gravid segments only, from the intestine of *Pycnonotus tricolor tricolor*; Kwango.

There was no difficulty in referring this worm to its genus, the uterus being typical. It was, however, quite impossible in the absence of a scolex to say to which species it belonged.

? Biuterina sp.

A fragment of a tapeworm without a scolex was obtained from the intestine of Caprimulgus fossii welwitschii; Kwango.

The segments were campanulate, and were so badly preserved that they showed no anatomical details except irregular genital pores and a paruterine organ. It was impossible to make a definite diagnosis of this fragment.

Genus III. Metroliasthes, Ransom, 1900

Metroliasthes lucida Ransom, 1900

Very numerous specimens from the intestines of Numida sp., Gallus gallus and Guttera edouardi; Kwango.

The majority of the worms measured up to 10 cm. in length, and the greatest breadth was about 2 mm. Most of the segments were much longer than broad, and in many of them the paruterine organ could be seen with the naked eye. The genital pores are irregularly alternate, and are situated near the middle of the lateral margin. The head is unarmed, and, as noted above, the paruterine organ in gravid segments is a very prominent structure.

The species has been recorded from various hosts, including *Numida* ptilorhyncha. It would appear that this is the first record of its occurrence in Guttera edouardi.

Family 5. PROTEOCEPHALIDAE La Rue, 1911 Genus *Ophiotaenia* La Rue, 1911

Ophiotaenia punica (Cholodkovsky, 1908) La Rue, 1911

Two specimens of this species were obtained from the intestine of *Causus rhombeatus*; Thysville. The species has previously been recorded from this host in Freetown, Sierra Leone.

Ophiotaenia congolense sp. nov.

Several specimens from the intestine of *Boodon olivaceus* and *Boodon lineatus* from Boma; and from three undetermined snakes, all of the same species, one from Boma and two from Malela.

The worms measure up to 8 cm. in length and have a maximum breadth of 2 mm. The scolex is slightly wider than the long neck. The gravid segments are longer than broad. The genital pores are irregularly alternate, and are situated at the middle of the lateral border of the segment.

The testes are oval bodies, arranged in two long lateral fields. They lie internal to the vitelline glands, with their long axes at right-angles to the long axis of the worm. There are about 30 on the poral side and 35 aporally. The cirrus sac, which is pyriform, occupies less than a quarter of the breadth of the whole segment, and from it the vas deferens continues to the mid-line.

The ovary is compressed against the posterior margin of the segment, and extends almost the whole width of the segment; it is bilobed and flattened; the lateral extremity of each lobe breaks up into several short branches. There

is a small well-defined globular shell-gland lying just posterior to the middle of the ovary. The vagina lies either anterior or posterior to the cirrus sac. When anterior, it usually crosses the vas deferens near the centre of the segment, but in a few segments it crosses the cirrus pouch. The vitelline glands are very numerous, lying in two long lateral fields between the sub-cuticular layer and the testes (fig. 8, A). The uterus begins in the form of a central stem, and

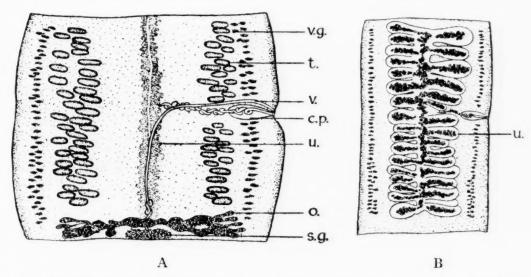


Fig. 8. Ophiotaenia congolense sp. nov. A.—Mature segments (\times 66). B.—Gravid segment (\times 26).

in the gravid segment has from 15 to 20 lateral branches on each side (fig. 8, B). The eggs are globular and measure 15μ in diameter.

Diagnosis. This species appears to be very closely related to Crepido-bothrium (Ophiotaenia) fima Meggitt, 1927, Crepidobothrium (Ophiotaenia) fixa Meggitt, 1927, Ophiotaenia mönnigi Fuhrmann, 1924, and Ophiotaenia nattereri Parona, 1901. The following table shows the relationships of the above species to ours.

	Genital pore	Testes	Uterine diverticula	Vagina
O. nattereri	A little anterior to middle	80–100	15-20 each side	Anterior or posterior (never crossing)
O. mönnigi	Middle	80	50-57	Anterior to cirrus
C. fima	Anterior to middle	68–89	27-33 ,,	Anterior or posterior (never crossing)
C. fixa	Near middle	71-94	20–24 "	Anterior or posterior (never crossing)
O. congolense sp. nov.	Middle	65	15–20 ,,	Anterior or posterior (crossing)

The classification of the genera normally included in the family Proteocephalidae (Icthyotaeniidae) has been considered at great length by La Rue (1914), Beddard (1913), Nybelin (1917), Woodland (1925), Meggitt (1927a) and Rudin (1917).

According to Nybelin (1917), *Ophiotaenia* La Rue, 1917, is synonymous with *Crepidobothrium* Monticelli, 1889. Woodland and Meggitt agree on this point, but Fuhrmann retains the genus *Ophiotaenia*.

SUPERFAMILY II. **Dibothriocephaloidea** Stiles, 1906 FAMILY DIBOTHRIOCEPHALIDAE LUEHE, 1902 Genus I. *Bothridium* Blainville, 1824

Bothridium pithonis Blainville, 1824

Seven specimens, one without a head, from the intestine of *Python sebae*; Malela.

The specimens were in every way typical and call for no comment, except that the head was contracted antero-posteriorly in such a way that the two tubular bothridia were globular in appearance.

As far as we are aware, this is the first record of this parasite from the African python.

Genus II. Duthiersia Perrier, 1873

Duthiersia elegans Perrier, 1873

Fragments, including a head, of this species were obtained from the intestine of *Varanus niloticus*; Malela and Thysville.

Woodland (1938) states that there are two species from Africa, namely D. elegans Perrier, 1873, and D. robusta Woodland, 1938. He also points out that Duthiersia fimbriata 'has never been described, and in consequence it is impossible to say which of the two (possibly three) African species I have recognized is its representative. Duthiersia fimbriata, therefore, automatically becomes a nomen nudum.'

Genus III. Lytocestus Cohn, 1908

Lytocestus adhaerens Cohn, 1908

Over 50 specimens from the intestine of Clarias sp.; Kwango.

The largest worms were gravid, and measured about 1 cm. in length by 4 mm. in breadth. The smallest, which were immature, measured about 2 mm. by 1 mm. They varied in shape within wide limits. Most of the larger ones, however, had the typical club-shaped appearance. The cuticle in all specimens tended to be rugose. The genital apertures are situated close to the

posterior extremity. The muscular system consists of two very definite layers; the outer layer lies internal to the nuclear layer of the sub-cuticle; the internal longitudinal muscles are much more strongly developed and separate the testes from the vitelline glands, i.e., the vitelline glands lie between the two layers of longitudinal muscles. The vitelline glands are therefore situated in the cortex. They have an annular arrangement, but are more prominent along the lateral margins; they do not extend on either side posterior to the ovary (fig. 9).

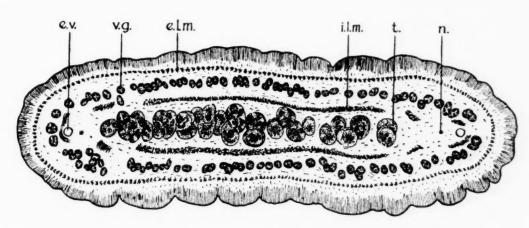


Fig. 9. Lytocestus adhaerens. Transverse section (× 44).

The uterine eggs measure 80μ by 30μ .

Diagnosis. Fuhrmann and Baer in 1925 reclassified the genera related to Caryophyllaeus and erected a new genus, Monobothrioides.

Woodland (1923) divided the family Caryophyllaeidae into four genera, namely:

- 1. Archigetes Leuckart, 1878. Including forms which possess a caudal appendage and into which, accordingly, our species cannot be placed.
- 2. Wenyonia Woodland, 1923. Including those Caryophyllaeidae in which, amongst other characters, the genital pores are situated in the anterior half of the body. As the genital pores in our species are placed posteriorly, they cannot be referred to this genus.
- 3. Caryophyllaeus O. F. Muller, 1787. This genus includes all species in which:

(a) The genital pores and ovary are posterior.

- (b) The vitelline glands lie internal to the internal longitudinal layer of muscles, i.e., the vitellaria are in the medulla.
 - (c) The vitellaria extend behind the ovary.

(d) Species parasitic in Cyprinidae.

Our species cannot be referred to this genus because the vitelline glands lie in the cortex and do not extend posterior to the ovary.

4. Lytocestus Cohn, 1908. This genus includes all those species in which:

(a) The genital pores and ovary are posterior.

(b) The vitelline glands lie between the outer and inner layers of longitudinal muscles, i.e., they are situated in the cortex.

(c) The vitellaria are wholly absent behind the ovary.

(d) Species parasitic in Siluridae and Mormyridae.

In our species, the muscular system resembles that of *Lytocestus*; the vitelline glands lie in the cortex and do not extend posterior to the ovary, and the parasites are from Siluridae.

Hunter (1927) makes the following remarks:

'At the present time this genus constitutes a refuse pile into which are cast many questionable forms. Some belong here without question. Such for example is the case of L. filiformis (Woodland 1923). Fuhrmann and Baer (1925) placed this form in the genus Lytocestus, along with L. chalmersius (Woodland 1924). These authors state at the time that while the descriptions of both forms are inadequate they clearly belong to the genus Lytocestus. However, from reading a description of L. chalmersius this author feels very strongly that a restudy of the parasite in question may lead to its being placed in the new genus created by Fuhrmann and Baer (1925), Monobothroides. In the first place, the type of scolex is the same in both forms, as each possesses a terminal introvert and a number of furrows extending longitudinally. Further than this the arrangement of the reproductive organs appears essentially similar, so that this author feels confident that it really belongs in the genus Monobothroides. The writer has therefore tentatively placed this form in the aforementioned genus.'

Baylis (1928) pointed out that the subfamily Lytocestinae contains the genera *Lytocestus* Cohn, 1908, *Monobothrioides* Fuhrmann and Baer, 1925, and *Capingens* Hunter, 1927. In the two former genera, postovarian vitellaria are absent. Our species, therefore, must belong to one of the first two genera. As the scolex does not bear longitudinal furrows or possess a terminal introvert, we refer it to the genus *Lytocestus*.

We are unable to find any points of difference between our specimens and L. adhaerens Cohn, 1908, and we therefore consider it to be this species.

SUPERFAMILY III. Lecanicephaloidea SOUTHWELL, 1930 FAMILY LECANICEPHALIDAE BRAUN, 1900 Genus Lecanicephalum Linton, 1890

Lecanicephalum peltatum Linton, 1890

One specimen only of a worm undoubtedly belonging to the genus *Lecanicephalum* was obtained from the intestine of *Zygaena malleus*, the hammer-headed shark; Malela.

The head was in poor condition, and, in order that a satisfactory examination might be made, it had to be destroyed. It was found to consist of two flattened, circular, disc-like plates, on the posterior one of which were found three suckers; apparently the fourth had been torn away with a portion of the scolex. The anatomy of a mature segment agreed in detail with that described by Linton

(1890) for this species, even to the presence of slender bristle-like spines on the cirrus.

A large number of detached segments were found in the phial. It is known that in tapeworms of this family, as well as of certain other families, the mature segments become detached from the strobila, are passed in the faeces, and live a free existence in sea-water, in which they become gravid.

PHYLUM NEMATHELMINTHES VOGT (quoted by Carus, 1863) CLASS ACANTHOCEPHALA RUDOLPHI, 1808 FAMILY GIGANTORHYNCHIDAE HAMANN, 1892 Genus Empodius Travassos, 1916

Empodius taeniatus (von Linstow, 1901)

Synonyms: Echinorhynchus taeniatus von Linstow, 1901 Echinorhynchus segmentatus de Marval, 1902

A very large number of these 'thorny-headed' worms were obtained from the intestine of *Numida* sp. and *Guttera edouardi*; Kwango.

PHYLUM ARTHROPODA
CLASS PENTASTOMIDA
FAMILY LINGUATULIDAE SHIPLEY, 1898
SUBFAMILY POROCEPHALINAE SAMBON, 1922
Genus I. Porocephalus Humboldt, 1811

Porocephalus clavatus (Wyman, 1845) Sambon, 1910

Two adult specimens from the intestine of *Psammophis notostictus*; Boma. The specimens measured about 1 cm. in length and 2 mm. in breadth. The body was composed of about 33 annuli. The outer pair of hooks surrounding the mouth were bifurcated. The species has previously been recorded from an unknown snake from Leverville, Congo, and from *Causus rhombeatus*, Gold Coast.

Genus II. Armillifer Sambon, 1922

Armillifer armillatus (Wyman, 1847) Sambon, 1922

Two specimens from *Python sebae*; Malela. There are four simple hooks on the head, and the body is composed of about 14 annuli. The species, both nymphs and adults, have been recorded on many occasions from various parts of Africa, the nymphs from the liver of man and from the peritoneal cavity of chimpanzees, and the adults from the lungs of *Bitis masicornis* and *Cerastes* sp. from Kumasi. It is interesting to note that a species of nematode of the genus *Amplicaecum* (apparently new) was found to have penetrated into, and to be lying coiled in, the head and part of the body of one linguatulid.

HOSTS

The following is a list of hosts examined, showing the parasites obtained from each.

from each.			
Ноѕт			PARASITE
	Class	MAN	IMALIA
	Order	PRI	MATES
Cercopithecus neglectus			Bertiella studeri
	Order	ROL	DENTIA
Arvicanthis striatus			Raillietina (R.) trapezoides
Funisciuris congicus			Catenotaenia lobata
Lophuromys sp			Hymenolepis sp.
Mastamus saucha			Catenotaenia pusilla
Mastomys coucha	• •	• •	$Catenotaenia\ pusilla$ $Raillietina\ (R.)\ madagascariensis$
Pelomys frater			Hymenolepis diminuta
			(Hymenolepis diminuta
Rattus rattus			Hymenolepis fraterna Cysticercus fasciolaris
			Cysticercus fasciolaris
Steatomys pratensis			Hymenolepis diminuta
Thryonomys swinderianus			Raillietina (R.) gracilis
Ord	er AC	CIPIT	CRIFORMES
Family AQUILIDAE	110		
Milvus tenebrosus aegyptius			Idiogenes flagellum
Orde	er CHA	RAD	RIIFORMES
Family GLARIOLIDAE			
			(Oligorchis kwangensis sp. nov.
Galachrysia nuchalis nuchalis	• •		∫ Oligorchis kwangensis sp. nov. ∵ { Haploparaxis crassirostris
Family OTIDIDAE			1 1
Lyssotis melanogaster	• •		Idiogenes otidis
Family ROSTRATULIDAE			3
•			(Dilepis irregularis sp. nov.
Rostratula benghalensis		• •	{ Dilepis irregularis sp. nov. { Hymenolepis spinosa
0.	rdor Cl	CON	IIFORMES
Family Ardeidae	dei Ci	CON	HTORWES
2			Hymenolepis unilateralis
-			
	der CC	OCCY	GIFORMES
Family Cuculidae			
Centropus superciliosus loanda	e	• •	Zschokkeella guineensis
Family Musophagidae			
Corytheola cristata			Raillietina (R.) undulata

Host

PARASITE

Order COLUMBIFORMES

Family COLUMBIDAE

Columba livia ... Raillietina (F.) korkei

Vinago calva calva Hymenolepis sp.

Order CORACHFORMES

Family BUCEROTIDAE

Bycanistes sp. .. Bertiella pinguis

Family CAPRIMULGIDAE

Caprimulgus fossii welwitschii ... ? Biuterina sp.

Family MEROPIDAE

.. Biuterina meropina var. Merops nubicoides

macrancristrota

Order GALLIFORMES

Family GALLINAE

Raillietina (R.) tetragona Gallus gallus ... Metroliasthes lucida

Family NUMIDAE

Numida sp.

Metroliasthes lucida Guttera edouardi

Empodius taeniatus

(Raillietina (R.) echinobothrida

Metroliasthes lucida Empodius taeniatus

Order PASSERIFORMES

Family DICRURIDAE

Dicrurus modestus coracinus ? Hymenolepis fringillarum

Family LANIIDAE

Dryoscopus angolensis angolensis Biuterina meropina var.

macrancristrota

Tshagra senegala rufofusca Anonchotaenia bobica

Family PLOCEIDAE

Spermaspiza haematina pustulata Echinocotyle rosseteri

Family Pycnonotidae

Astimastillas falhensteini Biuterina cylindrica Balopogon indicator indicator . . Hymenolepis stylosa

Nicator vires ... Raillietina sp.

Biuterina sp. Pycnonotus tricolor tricolor Moniezia?carrinoi

Ноѕт		DUO	ENICO	DTE	PARASITE
T '1 I	Ordei	r PHOI	ENICO	PIE	RIFORMES
Family IBIDAE Ibis ibis					Hymenolepis varicanthos sp. nov.
		Order	PICIF	ORN	MES
Family CAPITONIDA	AE				
Melanobucco bidente		dmanni			Raillietina (P.) bomensis sp. nov.
Melanobucco minor Family PICIDAE			••		Hymenolepis sp.
Campethera caroli c	aroli				Raillietina sp.
Campethera permist			••		Raillietina (R.) permista sp. nov.
		Order S	STEGA	NOI	PODES
Family PLOTIDAE					
Anhinga rufa rufa	• •	• •	• •	٠.	Echinorhynchotaenia tritesticulata
		Cla	iss RE I	PTIL	IΑ
		Orde	r SQU	AMA	ATA
*					(Lizards)
	laris nig	grolineat	tus		Oochoristica agamae
Varanus niloticus	• •		• •		Duthiersia elegans
	Subord	er Run	PTOGLO	SSA (Chameleons)
Chamaeleon etienii				•	Oochoristica agamae
Chamaeteon ettenti	• •	• •	• •	• •	Cochoristica agamae
	Su	border	OPHID	IA (S	nakes)
Boodon lineatus					Ophiotaenia congolense sp. nov.
Boodon olivaceus					Ophiotaenia congolense sp. nov.
Causus rhombeatus					Ophiotaenia punica
Psammophis breviros	tris				Oochoristica agamae
Psammophis notostici	us				Porocephalus clavatus
Psammophis sibilans					Oochoristica agamae
Pathon schoo				S	Armillifer armillatus Bothridium pithonis
Python sebae				٠٠ ٢	Bothridium pithonis
Snake sp	• •	• •	• •	• •	Ophiotaenia congolense sp. nov.
		C	lass PI	SCES	
Clarias sp					Lytocestus adhaerens
Zygaena malleus					Lecanicephalum peltatum

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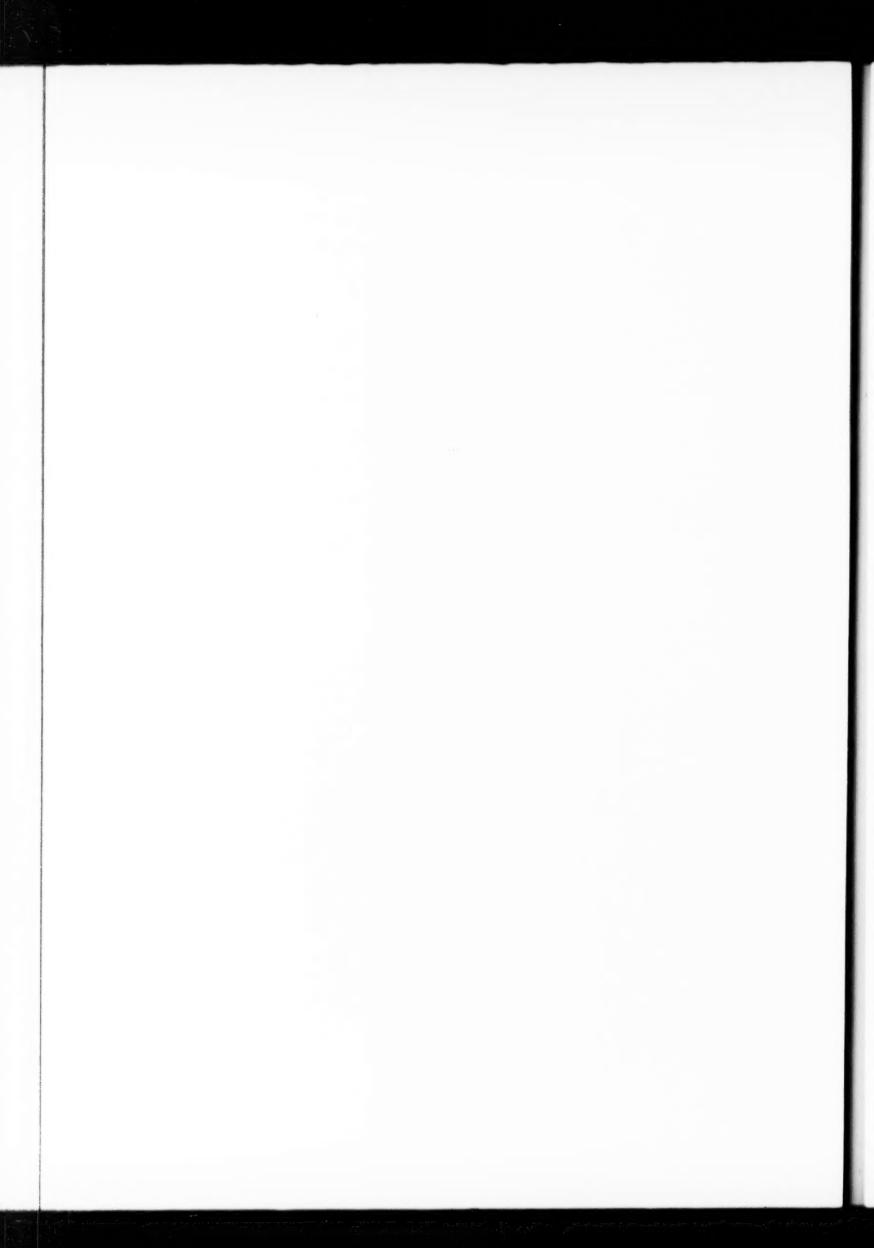
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(1938).



THE EPIDEMIOLOGY OF RELAPSING FEVER IN THE ANGLO-EGYPTIAN SUDAN

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I. INTRODUCTION

When relapsing fever reappeared in the Sudan during 1936 and 1937, after it had apparently died out completely two years earlier, the writer had an opportunity of observing the methods of spread of the disease, and of carrying out some experimental work with what appeared at first to be a new strain of spirochaete. The main features of this experimental work, and the conclusions reached, have been reported in previous communications (Kirk, 1938a, 1938b).

In the light of these conclusions, and of other information which was collected during the course of these investigations, it became apparent that the history of relapsing fever in the Sudan provided an epidemiological study of unique interest. The Sudan is surrounded by countries from which it is separated physiographically and in which relapsing fever is endemic. With the opening up of communications during the last 50 years, it has been invaded by relapsing fever at different times from the north, from the south, and from the west; but, although devastating epidemics may have resulted, all the available evidence suggests that the disease has never become established in endemic form in the Sudan. How far this is due to the energetic preventive measures which have been undertaken is difficult to estimate. With a European power in occupation

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of Abyssinia, the future is likely to see a further increase in communication across Africa from east to west, and it remains to be seen whether or not it will be possible under these circumstances to prevent the disease from becoming established in endemic form in the Sudan.

II. PHYSIOGRAPHY

The Anglo-Egyptian Sudan is bounded on the north by Egypt, on the east by the Red Sea, Eritrea and Abyssinia, on the south by the Belgian Congo, Kenya and Uganda, and on the west by French Equatorial Africa. It is of vast extent, and infinite in its variety, but for practical purposes it may conveniently be divided into two regions, the Northern Sudan and the Southern Sudan, by a line which corresponds approximately with the 400 mm. isohyet. The Northern Sudan has a typical desert climate, with low relative humidities and extreme variations in temperature, and is subject annually to a period of fierce desiccation lasting several months, while the more equable climate of the humid tropics prevails in the Southern Sudan. This division is more than a line of demarcation between two climatic zones: it separates, in effect, two regions which are entirely distinct from one another, in landscape as well as in climate, in their vegetation and fauna, and in their human inhabitants. It is not surprising, therefore, to find that the epidemiology of relapsing fever has been entirely different in these two regions.

The Gezira is a flat bare plain which lies in the triangle formed by the Blue and White Niles before they join at Khartoum. It contains about 5,000,000 acres, of which some 505,000 acres are irrigated with Nile waters from the Sennar dam. On this ground some of the finest cotton-crops in the world are produced, and from its share in the profits from the cotton the Sudan Government derives a large part of the revenues out of which the various social services of the country are maintained. Anything which produces adverse economic conditions in this area (e.g., a major epidemic dislocating the work in the cotton-fields) is therefore liable to affect the conditions which exist in the country as a whole.

III. THE PEOPLE

The Northern Sudan. The original peoples of the Northern Sudan appear to have been of mixed Hamitic-Negro stock, but from the seventh century onwards Arab invasion introduced a large measure of Asiatic blood and culture. Admixture has been going on for a very long time, and anything approaching pure racial types is rare. Minor superficial differences may have been produced by particular modes of life, but the Northern Sudan as a whole is Arabic-speaking and Islamic, and therefore culturally homogeneous.

The Southern Sudan, on the other hand, is inhabited by a bewildering variety of pagan negroid races, the majority of whom despise clothing and scorn Arabic and European cultures, preferring to live naked and unashamed in their

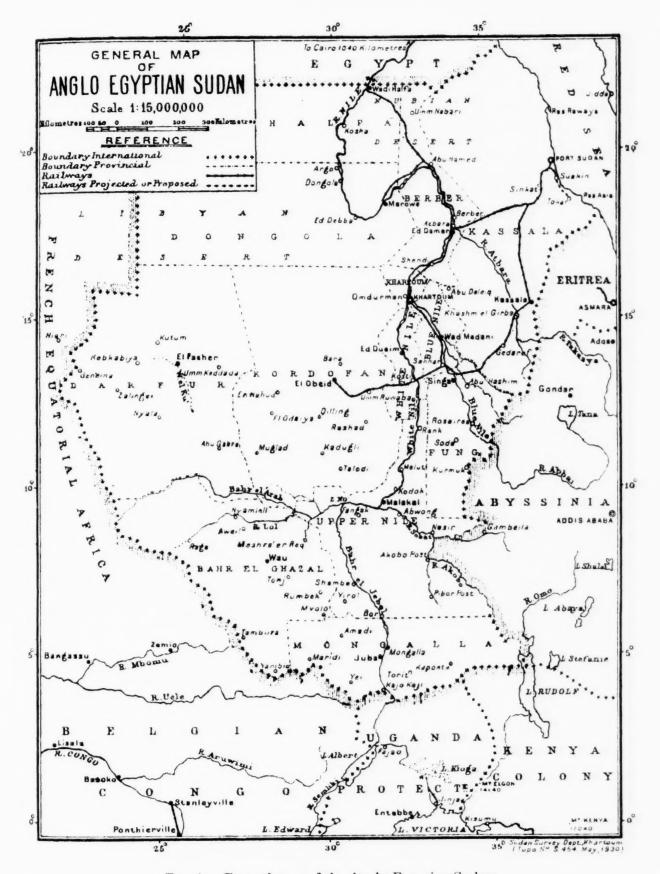


Fig. 1. General map of the Anglo-Egyptian Sudan.

own natural conditions, which are in some cases exceedingly primitive. It is of practical importance that in their nakedness these people have an efficient protection against louse-infestation and the diseases which follow in its train. Consequently, the southward spread of louse-borne relapsing fever is to a large extent limited by the habits of these peoples.

The movements of western peoples. The extreme west of the Northern Sudan is inhabited by peoples of negroid origin, who, although they have adopted Islam, remain comparatively untouched by the influence of Arabic language and culture. Their indigenous languages and folk-beliefs still flourish, and racially they are more akin to the peoples of French West Africa than to the inhabitants of the Northern Sudan proper. With the peoples of French West Africa they are often collectively grouped under the term 'Westerners', and conform to a common physical type, which can readily be distinguished from the other racial types of the Northern Sudan. The epidemiology of relapsing fever in the Sudan during the last 12 years has been very largely determined by the habits and movements of these peoples.

Formerly they were regularly raided by the Arab slave-traders, to whom they constituted an important source of wealth and man-power. Now, they travel long distances across the African continent in search of paid work, which will enable them to accumulate enough money to buy cattle and a wife and to settle down in their own countries. Every year there is a large influx of these Westerners into the Gezira for the cotton-harvest in November and December. Here, lacking the sophistication of the Arab, they make better labourers and earn good wages. After the cotton-picking season is over, they may return to their own countries if they have accumulated enough money. A few settle in various parts of the Sudan, others drift further east in search of work; their movements from place to place are affected by the demand for casual labour, but large numbers of them continue their eastward journey on the pilgrimage to Mecca. Their Mohammedan religion is curiously mixed up with the innumerable African superstitions on to which it has only recently been grafted, so that the pilgrimage is probably as big an incentive to the Westerners to leave their homes as the economic urge.

The total result is a slow, irregular, almost imperceptible, yet continuous, infiltrating migration of Westerners eastwards across the Sudan, and a smaller westward returning movement. The magnitude of this migration is difficult to estimate, but it is probably considerable. It may temporarily be deflected, accelerated or delayed by irregular changes in the labour markets, or by other political or economic events.

IV. HISTORICAL SUMMARY

Immediately prior to the British occupation in 1898, the Sudan passed through a long unhappy period of its history, during which famine, pestilence and war produced an extreme degree of depopulation (Cromer, 1906). Travellers

(Bruce, 1810; Burckhardt, 1822) and historians (Jackson, 1912; MacMichael, 1922) relate how different parts of the country were swept from time to time by epidemic diseases, which are variously described as plague, cholera and yellow fever. The application of these particular labels seems generally to have been quite arbitrary, and it is possible that some of these past epidemics may in reality have been relapsing fever; but the old descriptions are indefinite, and any retrospective diagnosis made after the lapse of more than half a century would be mere speculation.

Apparently the first cases of relapsing fever recorded from the Sudan were seen by Cummins (1910) in El Obeid in 1908. These were two Egyptian soldiers who had just returned from leave in Egypt, where the disease had already been described clinically by Sandwith (1905), and studied experimentally by

Graham-Smith (1909) and Dreyer (1910).

During the next two years Bousfield (1911) reported six cases seen in Khartoum—also Egyptian soldiers recently returned from Egypt. Writing with a knowledge of the medical conditions in the country at the time, Bousfield makes the interesting remark that 'human spirochaetosis is probably not endemic in the Northern Sudan, otherwise it seems scarcely possible that it would have missed recognition.' A year later Ranken (1912) recorded an isolated instance of latent spirochaetal infection in a monkey (Cercopithecus ruber) from the Southern Sudan, but the origin of this infection and any possible relation between it and human spirochaetosis are obscure.

In 1913 one case was found at Wadi Halfa on the Egyptian frontier, and one case in Khartoum, while in 1916 there was a small outbreak at Halfa—16 cases in all; the origin of these infections was traceable once more to Egypt. For the years 1917 to 1924 the records are scanty, but some 50 cases were observed during this period in and around Atbara. Bousfield had pointed out the danger of Egyptian immigrants being able to introduce the disease into the Sudan and so permanently infecting a district apparently free from human spirochaetosis. The distribution of the cases from 1908 to 1924 certainly suggested that the disease had a tendency to spread along the lines of communication between Egypt and the Sudan.

During 1925, however, there were no further cases in the Northern Sudan, but six cases were found in Mongalla, on the Uganda frontier. Tick-borne relapsing fever is prevalent in Uganda, and *Ornithodorus moubata* was found in some of the rest-houses which had been occupied by these cases. So it may be

presumed that on this occasion the disease was tick-borne.

It is probable, therefore, that until 1926 relapsing fever had not established itself in endemic form in the Sudan, nor had it, at any rate under British administration, assumed epidemic form.

In 1926 relapsing fever invaded Darfur from the west in epidemic form. Riding and MacDowell (1927) suggest that this disease originated from an endemic focus in the Marra mountains; but according to Atkey (1929) the

disease was introduced into the Sudan by immigrant labourers from the French Sudan, and there seems to be more support for this argument than for Riding and MacDowell's. Louse-borne relapsing fever first appeared in French West Africa in 1921, where, according to Kerrest, Gambier and Bouron (1922), it was introduced by repatriated Senegalese troops from Syria. Other observers, however, notably Nogue (1925) and Rigollet (1925), believe that this epidemic originated locally from some unknown endemic focus in West Africa, and produce some convincing arguments in support of their views.

However it arose, this was undoubtedly one of the biggest historical epidemics of relapsing fever. It spread unchecked across British and French West Africa into the Tchad region (Lasnet, 1930), and finally appeared in Wadai (Le Gac, 1931). The mortality in these regions is unknown: 128,750 deaths were estimated in Kano alone (McCulloch, 1925), and in other districts the

death-rate is said to have been even higher.

In September, 1926, the disease reached Darfur, the most westerly province of the Sudan, where it spread from village to village with amazing rapidity, and in the space of six months had killed over 10,000 people. It commonly swept through a village and infected half or a third of the population, with a casemortality of 70 per cent., after which its virulence abated (Maurice, 1932).

For four years the eastward spread of the disease into the central Sudan was prevented by a vigorous campaign, including the establishment of quarantine posts, the destruction of lice, and the treatment of the sick (Beveridge, 1928). Had this virulent disease invaded the Gezira, the work in the cotton-fields would have been seriously disorganized, and the whole Sudan might have been faced with an economic disaster of the first magnitude. During these four years the virulence and infectivity of the disease appeared to diminish, for the wide-spread epidemics which had decimated the population of Darfur died down and gave place to smaller, localized epidemics, of lesser virulence and infectivity. This decrease in virulence was accompanied by the appearance of mild subacute or ambulatory cases, extremely difficult to detect at the quarantines (Atkey, 1931). It was inevitable that sooner or later some of these cases would slip through, and pass with the stream of immigrant labour into the irrigated areas of the Gezira.

This happened in 1930. From April to August of that year sporadic cases occurred in various parts of the Gezira, but on August 30th six cases were reported in one locality, and from that time onwards fresh cases continued to occur, in spite of vigorous control measures, until June, 1931, when the incidence fell abruptly. A small recrudescence took place in the autumn of 1932, but this died out before the end of the year, and during the following three years the Northern Sudan was free from relapsing fever, although in 1934 an isolated case was found in Malakal, in the Southern Sudan. No definite evidence exists whether this infection was louse-borne or tick-borne, as no investigations were made.

V. RECRUDESCENCE OF THE DISEASE IN 1936 AND 1937

In 1936 relapsing fever appeared once again in the Sudan; in August of that year the first case was discovered in Singa, and during the remainder of the year a few more cases occurred in the same district. Enquiries about their movements revealed that the patients had recently come from Abyssinia. The cases and their contacts were promptly isolated and disinfested, and by the end of the year it appeared that the situation was well in hand, and that any tendency to epidemic spread had been prevented.

This unfortunately was not so. Sporadic cases began to occur during the early part of 1937, and by the end of March the total for the whole Sudan was 39. Infections were reported from places as far apart as Kassala (8 cases) and Fasher (2 cases); one case was found in Nahud, and the remainder were distributed between the Fung and Gezira districts of the central Sudan. Cragg (1922) describes how relapsing fever in India frequently appears to take origin simultaneously from multiple foci in different parts of the country, and it looked as

if the same thing were about to happen in the Sudan.

In August the incidence rose abruptly, and it was everywhere noticed that the disease, as in former years, was almost wholly confined to natives from the western Sudan and French West Africa. The possibility of another major epidemic was disquieting, expecially as it had been possible at a comparatively

early stage to incriminate the louse once more as the vector.

In spite of the almost complete restriction of the disease to natives of the western Sudan and French West Africa, it soon became apparent that the infection was this time coming from a new source, viz., Abyssinia and the other countries east of the Sudan. Although the existence of relapsing fever in Abyssinia has been recognized for many years, comparatively little is known of the conditions under which it occurs; a short summary of the available information has been given in a previous communication (Kirk, 1938a). The factors leading to the infection of the Sudan may in a general sense be described as part of the aftermath of the Italo-Abyssinian war; but it may definitely be stated that it was not the result of an influx of Abyssinian refugees, and an analysis of the factors which led to the introduction of the disease into the Sudan is not without interest.

After the military occupation of Abyssinia by the Italians, there still remained the longer process of pacification and development, implying money and labour. It is not surprising, therefore, to discover that a large migration of Westerners had been deflected into Eritrea and Abyssinia during the previous two years, in the reasonable expectation that they would there find a market where labour would command high prices. After they arrived in these countries, however, they found conditions disappointing. The food and climatic conditions were not those to which they had been accustomed. The work (mostly road-making) was hard, and, owing to the unsettled state of the country, had frequently to be carried out under trying circumstances. Rates of pay were

no higher than they could obtain elsewhere, and, owing to frontier restrictions on the export of currency, they very soon discovered that they were unable to take their accumulated earnings out of the country. Finally, according to their own statements, smallpox and fever (presumably relapsing fever) appeared in epidemic form, and many people died of these diseases. The result was that the Westerners began to leave the Italian colonies as rapidly as they could, and poured back into the Sudan in large numbers, intending to find work there or to return to their own countries.

On this occasion, therefore, the infection was introduced into the Sudan by an unusually large and rapidly moving westward migration of the same peoples who had introduced the disease in 1926 from the west. A few were clinically ill on arrival and were easily detected; but others arrived incubating the disease, or during an apyrexial interval, and these were more elusive, for once they reached the railway it was possible for them to travel long distances into the Sudan before they fell sick, thus leading to the appearance of the disease in localities far removed from the original source of the infection. Large numbers migrated to the Gezira in their search for work, and consequently it was there that the heaviest incidence of the disease occurred.

VI. THE VECTOR

Working with spirochaetes from some of the Egyptian cases, Balfour (1911) concluded that the louse was the vector; he was unable, moreover, to infect ticks (*Ornithodorus savignyi* and *Argas persicus*) with these spirochaetes. Riding and MacDowell (1927) showed that the Darfur epidemic was louse-borne, and Ingram (1924), working in West Africa with what was presumably the same strain of spirochaete, was unable to infect *Ornithodorus moubata*. The present writer was able to show that the Abyssinian disease which invaded the Sudan during 1936-37 was also louse-borne (1938a), but was unable to infect *Ornithodorus savignyi* or *Argas persicus* (1938b).

No experimental work was carried out in connection with the few cases which occurred in the Southern Sudan in 1925 and 1934, so that it is possible that these may have been due to tick-borne infections; those which occurred in Mongalla in 1925 almost certainly were. No views can be expressed as to the origin of the infection which was found in the markey.

the origin of the infection which was found in the monkey.

It has already been pointed out that the southward spread of louse-borne relapsing fever is likely to be limited by the freedom from louse-infestation which is enjoyed by the naked southern races. It is unlikely also that tick-borne infections will become established in the Northern Sudan, owing to the absence of a suitable vector. The distribution of *Ornithodorus* in the Sudan appears to be limited to *moubata* in the south and to the closely related *Ornithodorus* savignyi in the north. Although this tick has been regarded at different times as the probable vector of relapsing fever in Abyssinia (Wenyon, 1926), in British Somaliland (Drake-Brockman, 1915) and in Angola (Wellman, 1905), these

conclusions are based on general considerations only, and have not been substantiated by laboratory findings. It is true that under experimental conditions Ornithodorus savignyi can transmit all the varieties of relapsing fever transmissible by Ornithodorus moubata. But the infection is not passed on to the next generation in the case of Ornithodorus savignyi, and it is possibly for this reason, as Brumpt (1936) has suggested, that the latter tick has never been found naturally infected in any country. Batches of Ornithodorus savignyi from various parts of the Sudan have at different times been examined by Balfour (1911), Archibald (1910), Brumpt (1936), and by the present writer (1938b), but on no occasion has any evidence of spirochaetal infection been found.

The epidemiology of relapsing fever in the Northern Sudan can probably therefore be regarded as that of the louse-borne infection alone. Moreover, although the disease has been introduced at different times from three separate and distinct sources of infection, all attempts to infect ticks with the particular strain of spirochaete concerned on each occasion have been unsuccessful. As far as the Northern Sudan is concerned, therefore, no evidence can be found that these arthropods have played any part whatsoever in the epidemiology of relapsing fever.

VII. GENERAL EPIDEMIOLOGY

It is generally stated (Selwyn-Clarke, Le Fanu and Ingram, 1923) that epidemics of relapsing fever begin slowly and increase in virulence and infectivity. In the Sudan, the epidemic of 1926 began with explosive violence. By 1930 the virulent disease of 1926 had become a relatively benign infection, with a mortality of 10 per cent. instead of 70 per cent., and the appearance of healthy carriers whose infection was entirely subclinical although spirochaetes were readily found in their blood (Atkey, 1931). It is suggested that this epidemic died out during its passage across the Sudan, as epidemics frequently do (Currie, 1930), by progressive enfeeblement of the strain. There seems to be no evidence to connect this epidemic with the 1929 epidemic in the Djig region of Abyssinia (Bruns, 1937), nor with the recrudescence of the disease in the Sudan in 1936.

It is, of course, theoretically conceivable that the disease of 1936 might be merely a reactivation of the 1926 disease, which had meantime lain dormant in some reservoir, or in residual brain infections which might have become active again as a result of malaria or some other disease. No evidence can be obtained

to support these views.

The way in which unrecognized sporadic cases maintain the infection in Algeria during the periods between epidemics has been described by Sergent and Foley (1922). The possibility of this happening in the Sudan is difficult to exclude, and incidentally may provide a public health problem for the future. Yet it seems clear from our studies that, if it be assumed that this process occurred in 1936, it was of academic importance only, compared with the obvious heavy influx of infection across the eastern frontier. Similarly, in the face of a major

epidemic slowly spreading across Africa from the west, there seems no necessity to assume (in the absence of concrete evidence) the persistence of the old Egyptian disease in sporadic or endemic form to account for the epidemic of 1926. It is suggested that the view expressed by Bousfield (1911) is probably true as far as the Northern Sudan is concerned. Human spirochaetosis is not endemic, but has at various times been introduced from adjoining countries. During the period 1908-25 it was from Egypt, in 1926 from French West Africa, and in 1936 from Abyssinia and Eritrea. The disease in 1925 may or may not have been an introduction from Uganda.

The classical epidemiological associations of relapsing fever—war, civil unrest, economic stress, etc.—have been shown clearly at work in the propagation of the 1936 disease. Their influence is less clear in previous epidemics. It would be easy to invoke the world-wide economic depression of 1929-30, which hit the Sudan badly, in explanation of the disease in the Gezira in 1930; but it is doubtful if this would be justified. The evidence all suggests that this was the tail-end of the eastward spread from Darfur of the 1926 disease, which began in West Africa in 1921. Attempts to define the factors underlying the spread of this disease across Africa during these particular years lead into realms of pure speculation.

VIII. INCIDENCE

Sex. As in India, West Africa, and other countries in which relapsing fever is found, the majority of cases in the Sudan occur in males, the actual incidence in cases personally observed by the writer being 98 per cent. males and 2 per cent. females. Except for the cases described in West Africa by Selwyn-Clarke, Le Fanu and Ingram (1923), this is a much higher percentage incidence in males than is generally observed.

This very large preponderance of the disease amongst males is not dependent on the degree of lousiness of the two sexes, for this infestation is shared in equal measure by both men and women. Indeed, women are often more heavily infested than men, owing to the habit of shaving the scalp almost universal among the latter, while elaborate coiffures are favoured by the women. The true explanation lies in the fact that the infected males belong almost exclusively to immigrant tribes from the west, who had come to the Sudan, or to Abyssinia and Eritrea, in search of work and money. It is contrary to the traditions and habits of these people to bring their womenfolk with them under these circumstances, especially if there is any question of proceeding further on the pilgrimage to Mecca.

Age. As the majority of cases occur among immigrant males from the west, it follows that most of the cases will be adults, since the journey across Africa to Eritrea and Abyssinia, or even to the Gezira, is not a thing to be undertaken lightly by other than healthy adults. In cases observed by the writer the ages

varied from 15 to 55 years, but the greater proportion fell into the 20-35 agegroup. (Ages were assessed approximately on the physical appearance of the

patients.)

Race. A very pronounced racial incidence is observed with regard to relapsing fever in the Sudan, since over 90 per cent. of the infections occur among those races which are grouped together under the collective term of 'Westerners'. This may, indeed, be described as the most striking epidemiological feature of the disease in the Sudan, and has been so ever since the 1926

epidemic invaded Darfur.

Various factors combine to produce this striking racial incidence, particularly in the 1936 disease. To begin with, these peoples, being immigrants from the infected countries, would naturally be the first to contract the disease. Once infected, their habits and traditions, together with the conditions under which they had been living, would ensure a ready spread of the disease. Owing to the scarcity of water in their home districts, except during the wet season, these people do not wash very frequently. Their standard of personal cleanliness is below that of the riverain peoples of the Sudan, who have more convenient opportunities of washing, and who are, on the whole, subject to a much less heavy louse infestation.

In respect of physical conditions, the plight of the immigrants observed by the writer during 1936 and 1937 was little different from that of refugees. They were dirty, tired, harassed; their homes were in another land, and they possessed no house-property or relatives with satisfactory living-accommodation in the districts they passed through. For months they had probably been living on a very inadequate diet, as the most constant complaint against the conditions

which they had experienced was 'al akl wis'han '-the food was bad.

Season. In the Sudan a definite tendency can be observed in the incidence of relapsing fever to rise sharply in August and September, i.e., during the latter part of the rains. A similar tendency to seasonal prevalence of relapsing fever has been observed in India (Gill, 1922; Cragg, 1922), where it has been correlated with hot humid weather; in Algeria (Sergent and Foley, 1922), where it has been correlated with the cold winter months; in Abyssinia (Bruns, 1937), where it seems to depend on the rains; and in other countries. In China, Shrimpton (1936) points out that the seasonal incidence is liable to be masked or disturbed by epidemics, and the same occurs in the Sudan. Fig. 2 shows graphically the relation between relapsing fever and rainfall in the Blue Nile province of the Sudan, where the most accurate figures are obtainable.

Cragg (1922) has drawn attention to the effects of different temperatures and humidities on lice, and it is probable that in India and other places where the disease is endemic this may be one of the most important factors which determine its seasonal prevalence. In the Sudan, however, there is another factor which must also be considered, viz., immigration: this is probably of at least equal importance. It has already been described how the ripening of the

cotton-crop, bringing its annual demand for labourers, will cause an influx of Westerners at this time. The actual harvest takes place in November and December, and the largest influx occurs at this time. During September and October, work begins on a smaller scale, with various minor weeding, 'hishing'

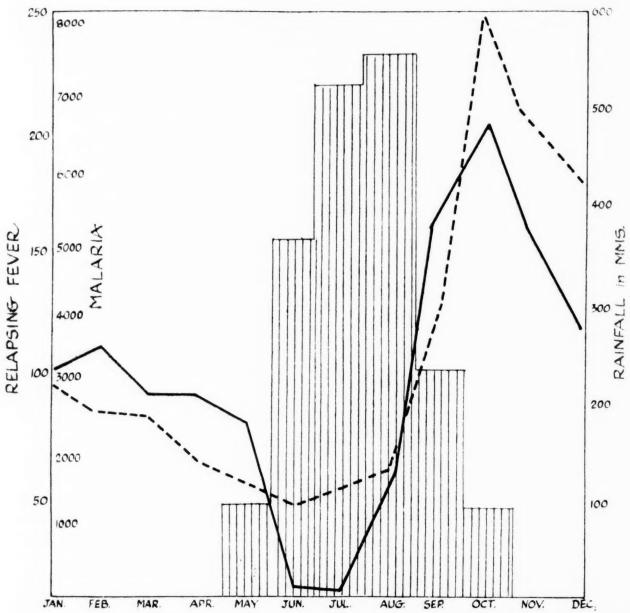


Fig. 2. Diagram showing the seasonal incidence of relapsing fever, malaria and rainfall in the Blue Nile province. The continuous line indicates the number of cases of relapsing fever, the interrupted line the cases of malaria, and rainfall is represented by the histogram. The relapsing fever values for each month are the summation of the figures for each January, February, etc., during the period 1930-37, while the values for malaria and rainfall are averages, taken over the same period.

and cleaning operations, and at this time there is a smaller preliminary influx of Westerners. This close correlation between the seasonal movements of immigrant labourers and the seasonal incidence of relapsing fever is probably of more significance than any direct relation with meteorological variations.

IX. RELATION TO OTHER DISEASES

Malaria. The diagram in fig. 2 shows the seasonal incidence of relapsing fever compared with that of malaria in the Blue Nile province. It will be observed that there is a fairly close correspondence. It is practically axiomatic that the seasonal incidence of malaria in the Northern Sudan is determined by the distribution of the rains throughout the year.

Typhus. Sergent and Foley (1922) have drawn attention to the close association between epidemics of louse-borne relapsing fever and louse-borne typhus in Algeria, where the two diseases have the same seasonal prevalence, the same general course, and commonly either follow one another or coincide.

No such association occurs in the Sudan, where typhus is unknown. It has often been a matter of surprise that clinical typhus has never been reported in the Sudan, although from time to time various cases of suspicious fever have been noted. There is no real clinical evidence that any of these have been *Rickettsia* infections, and the few sera which it has been possible to obtain from such cases have been negative. In order to determine whether this absence of typhus is real or merely apparent, a survey was carried out during 1937, by means of the Weil-Felix reaction, on a large number of human sera and sera from wild rats (Horgan, 1938). With the exception of two doubtful human sera, the results were completely negative in 1,000 human sera and 240 rat sera, and appear to confirm the clinical experience that typhus is unknown in the Sudan.

Plague. Like typhus, plague has never been reported clinically in the Sudan. Cragg (1922) has pointed out the inverse seasonal prevalence of plague and relapsing fever in India, and from this inverse relationship he was able to deduce in retrospect that an abnormally high peak in the 'deaths from fever' curve was due to an unrecognized epidemic of relapsing fever. A glance at the monthly malaria incidence graph (fig. 2) will show the fallacy of drawing such a conclusion in the Sudan.

Nicolle and Anderson (1927) have suggested that the relapsing fevers originated as infections of small rodents. It is a very striking epidemiological coincidence, and one which has interested the writer for some time, that three of the principal infections of rodents transmissible to man are unknown in the Northern Sudan. Reference has been made above to plague and typhus, and the present writer has elsewhere drawn attention to the apparent absence of leptospirosis (1938c).

X. MORTALITY

The mortality among the cases observed during 1936 and 1937 was 12 per cent. This compares with a mortality of 12 per cent. during 1930-31 in the Gezira, and a mortality of 70 per cent. in Darfur in 1926-27. It is noteworthy that in a small outbreak of 60 cases which occurred in Kuttum (Darfur) in 1937 the mortality was 30 per cent., and a similar discrepancy was noted in 1930-31 between cases in Darfur, where the mortality was 40 per cent., and those in the Gezira, where it was only 12 per cent.

It is difficult to explain this discrepancy on the grounds of racial or dietetic differences in the provinces concerned. In both instances it is the same races which were affected, and according to their own account the food they had lived on, at least during 1936 in the Italian colonies, compared unfavourably with that in their own countries in the west. The most probable explanation is that it is due to the way in which medical attention is hindered by transport difficulties in a rural area like Darfur, as compared with the highly organized Gezira and the places near the railway line. Probably, therefore, the Darfur mortality is much nearer what the figure for the untreated disease would be, and suggests that in the absence of proper medical and sanitary measures this disease might very well repeat the havoc of former years.

XI. PREVENTION

As far as the Northern Sudan is concerned, all the evidence which it has been possible to obtain incriminates the louse, and the louse alone, as the vector. The principal indication for prevention is, therefore, the destruction of lice. This is easily enough accomplished in individual cases, but its mass application to an infected population which is subject to the effects of immigration, and to the movements of people in search of work, is by no means easy. A detailed discussion of the administrative and other difficulties which have to be overcome is outside the scope of this present work, but Atkey (1931), in this connection, has already pointed out the immense value of the properly organized and well-linked dispensary system which forms an integral part of the present medical administration of the Sudan.

Experience in the Sudan has shown that epidemic louse-borne relapsing fever is controllable, if sufficient effort is made, and that the ultimate effort required is inversely proportional to the rapidity with which control measures can be made effective at the beginning of an epidemic. Under present conditions it is believed that the chances of any epidemic suddenly assuming major proportions throughout the country are remote; but a greater risk at the present time seems to be that the disease may become established in endemic form. With the further pacification and colonization of Abyssinia, the future is likely to see a further increase in traffic across the Sudan from infected countries in the west to infected countries in the east, and *vice versa*. Whether it will still be possible under these circumstances to prevent the disease becoming endemic among the population of the Sudan remains to be seen.

XII. SUMMARY

1. The history of relapsing fever in the Sudan is summarized, and certain relevant physiographical, racial and economic features of the country are noted.

2. Louse-borne relapsing fever was introduced into the Sudan during 1908-24 from Egypt, in 1926 from French West Africa, and in 1936 from Italian East Africa.

3. The infection of the Sudan in 1936 and 1937 from the east was a consequence of the Italo-Abyssinian war, although it was not effected by Abyssinian refugees.

4. There is no evidence that louse-borne relapsing fever exists in endemic form in the Sudan. Each time the disease has appeared, its origin has been

traceable to infected immigrants from adjoining countries.

5. During the last 12 years the incidence of the disease has been confined almost entirely to adult male 'Westerners', i.e., negroid immigrants from the Western Sudan and French West Africa.

6. The disease shows a tendency to seasonal incidence at the end of the This is correlated with the seasonal movements of immigrant labourers rather than with meteorological variations.

7. Although a close association has been observed elsewhere between epidemics of louse-borne relapsing fever and louse-borne typhus, no such relation exists in the Sudan, where epidemic typhus is apparently unknown.

8. Infections in the Southern Sudan may be tick-borne, but there is no evidence that these arthropods have played any part in the epidemiology of relapsing fever in the Northern Sudan.

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NOTES ON SIPHON ACTION, WITH SPECIAL REFERENCE TO ANTI-MOSQUITO WORK

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The purposes for which large water-siphon systems can be used are various. In recent years siphons have been employed in anti-mosquito work, particularly in Malaya, where Scharff, de Villiers, Williamson and others have developed their use. If sufficient fall can be obtained, it is possible, by means of a siphon, to produce a rapid transient rise of the water-level in a stream in which anophelines are breeding. In order to produce this effect, a dam, with a siphon incorporated, is built on the stream above the breeding-places; the reservoir formed is emptied periodically by the siphon. The volume of water projected suddenly into the stream-bed immediately below the reservoir is carried downstream in the form of a wave. This wave, which may be small, produces several effects, one or all of which may destroy the anophelines. water may carry eggs, larvae and pupae to such a distant position lower downstream that they will no longer constitute a danger, even if they survive and the adults finally emerge. Or the rising water may leave them high and dry, stuck on vegetation or adhering to the banks above the normal water-level. The turbulence set up by the action of the siphon may cause fine gravel, silt and clay to mingle with the water. This suspended material may kill the larvae by bodily distortion, or, if the clay is extremely fine, by occlusion of the spiracles and consequent asphyxiation.

One requirement of siphons, where flushing by them proves efficient in anti-mosquito work, is that their action should be automatic. Such siphons eliminate manual labour and the expense of oiling in accessible streams, and render possible the control of stretches of ravine not easily accessible for oiling. It is too early to say what the disadvantages may prove to be, but erosion of the bed of the stream and of its banks has to be considered. There is also the possible effect, where the sides of the stream are not properly sloped, that the intermittent rise of water may form pools beyond the normal margin of the stream; developing anophelines, dislodged from the stream, may survive there, or fresh oviposition may take place in the pools, thus establishing new breeding-places. Even where the siphon-method is practicable it may be found that, owing to the character of the ground, it is impossible to control the whole stream, because effective dams cannot be built at suitable points above all the breeding-places. In this case measures such as spraying with oil or Paris green must be applied to the uncontrolled stretches of water. The final disadvantage

to be mentioned is that the cost of installation, together with the maintenance of the edges and bed of the stream, may prove more expensive than other means. Only experience gained from trial and error on the spot can show whether the

method can be applied with success in any locality.

Siphons of large size have hitherto been built in connection with hydroelectric schemes and in dams impounding water intended for public supplies or irrigation. The function of the siphons in such cases is to deal rapidly with an excess of water, which would raise the level to an undesirable distance above the weir over which the normal surplus of water is being discharged. By the aid of a sufficient number of large siphon spillways any extra flood-water can be discharged downstream so rapidly that the water-level above the weir is kept at a convenient prearranged height.

There are two situations in which some engineers look with favour on the introduction of siphon spillways. The first is where great expense would be involved in making a dam strong enough to resist the pressure of an exceptionally high water above the weir; this condition is apt to be found in hilly country with little vegetation and much underlying rock, both conditions tending to a rapid run-off of rain-water. The second is where it is impossible to permit the weir to be raised above a given height, owing to the danger of flooding land; this condition is present where the country beside and below the dam

is low-lying.

A clear distinction exists between the functions of siphons which are to be used for the purpose of spillways and those which have hitherto been used as part of a system of anti-mosquito measures. This difference of function entails differences in size and in the methods of construction and operation. In the former case, what is required is that, when the water—e.g., in a large reservoir—reaches a certain dangerous level, the siphons will at once come into operation automatically and continue to siphon the water away until such time as the level in the reservoir has been reduced to the safety-mark. In the latter case, the object usually is to empty a relatively small reservoir as frequently as may be desired and as completely as possible, its total water-contents being poured suddenly downstream.

It is possible, however, that large siphons may be found of service for anopheline control in special conditions. For example, where the bulk of the water of a river is diverted for hydro-electric or other works, the old river-bed with pools of water may be a serious source of breeding. The construction of dams, with siphons in them, at intervals, would keep the water at a steady level which would only be altered periodically when the siphons acted.

Many names are applied to the various parts of the siphon; the terminology

employed in fig. 1 is that used by Naylor (1935).

Useful hints as to the possibilities of siphons may be obtained in the laboratory from experiments with simple apparatus. For example, three glass tubes were obtained about 12 inches in length and $\frac{1}{4}$, $\frac{1}{2}$ and 1 inch in internal

diameter. They had been given a smooth bend in such a way as to provide two limbs more or less parallel, one being longer than the other by about 3 inches. The tube with the internal diameter of $\frac{1}{2}$ inch was used for most of the first observations. In preliminary experiments with the tube of $\frac{1}{4}$ -inch internal diameter, it was found difficult to obtain an inflow to the siphon reservoir so slow that it did not cause siphon action to take place as soon as the water reached the crest-level. On the other hand, it happened that, with the 1-inch internal diameter siphon, the full flow of the particular bench-tap with which the experiments were done was not sufficient to provide a 'fast' inflow to the reservoir; the rates of inflow, fast, medium and slow, are defined below.

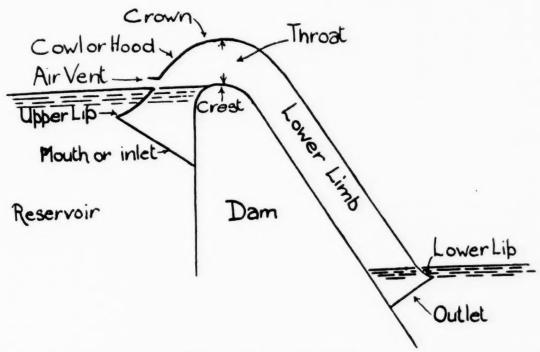


Fig. 1. Diagram of siphon.

A tin box, 4 inches by 4 inches by 7 inches deep, was used as the reservoir; in one side, taken to represent the dam, a circular aperture was cut at some distance below the top margin. The shorter limb of the $\frac{1}{2}$ -inch glass siphon was thrust through the aperture and so arranged that it reached to within about half an inch of the bottom of the reservoir. The siphon was held in position by a mass of plasticine applied round the aperture in the box.

Results with open outlet. This apparatus was placed on the bench beside a sink, with the long limb projecting into it, but not submerged in water; a rubber tube was led from the bench-tap into the box, and it was possible to follow events during the time that the reservoir was being filled.

With any rate of inflow of water the first thing noted was that, as soon as the rising water in the reservoir has occluded the aperture of the inlet-limb of the siphon tube, a small difference in level was established between the water in the

reservoir and that inside the siphon. The level inside the tube was always higher than the reservoir-level. Three rates of flow into the reservoir were employed, fast, medium and slow. These were determined by the following results obtained with the model, when it was used with the outlet open.

Fast. The water in the inlet-limb, its level preceding that in the reservoir, rose to the crown, and the throat of the siphon filled and ran full bore. The water in the reservoir continued to rise till it overflowed the dam, showing that the siphon could not cope with the inflow. When the inflow was stopped the reservoir emptied, its water-level falling rapidly to the level of the inlet, with the

resultant admission of air into the siphon.

Medium. The water in the inlet-limb, its level preceding that in the reservoir, rose to the crown; the throat of the siphon gradually filled and then ran full bore; there was a considerable escape of water before siphonage occurred. The water-level in the reservoir was at once lowered by the siphonage to the level of the inlet, at which point air entered the inlet and broke the siphon action. The reservoir quickly filled up again and the whole procedure was repeated. This cycle tended to go on automatically, but only if enough air was admitted at the end of each action. Imperfect breaking often resulted in irregular siphoning and run-through of water. When the inflow was stopped, the reservoir emptied, the siphon lowering the water-level until air entered the opening of the inlet-limb.

Slow. The water in the inlet-limb, its level preceding that in the reservoir, rose to the crest. It began to flow over the crest and down into the sink. The rate of inflow could raise the level inside the throat to immediately below the crown. Provided, however, that the water did not rise above this level, it flowed out of the siphon as quickly as it flowed into the reservoir. No siphon discharge was set up and all the water ran to waste. When the inflow was stopped, the water-level in the reservoir fell a little, but it remained at, or just

below, the level of the siphon-crest.

The results obtained from the fast and the slow inflows were quite unsatisfactory for the purpose of siphoning as an anti-mosquito measure. The reason of this was that neither with the fast nor with the slow inflows was there any periodical discharge through the siphon, and there was great waste of water.

Sealing. In the above experiment, with the open-outlet siphon, there was much loss of water with all rates of inflow. The waste which occurs during fast flow may be largely prevented by special remedies mentioned later. With regard to the waste which occurs with medium and slow inflows, we may consider first the effect of sealing the lower end of the outlet as a means of preventing waste. It is possible to cause initial retention of water in a siphon reservoir if the outlet of the lower limb is adequately sealed.

A pad of plasticine applied to the outlet of the model prevented the water rising in the inlet-limb, and the removal of the pad, when the reservoir-level was high, produced siphonage. Using the 1-inch diameter model, siphonage occurred with certainty if the pad was removed when the reservoir water-level stood at the level of the crown or higher; it failed to occur when the water-level of the reservoir was at the crest or lower.

The use of a water-seal. If, instead of the pad of plasticine, a basin of water is employed for sealing the lower end of the outlet-limb, a minor degree of retention effect is produced. The results obtained depend on the depth to which the end of the outlet-limb is immersed in the water.

For example, on filling the reservoir, when the depth of immersion of the lower limb of the ½-inch glass model was only a quarter of an inch, bubbles of air began to escape from the immersed outlet, almost immediately after the inflow had occluded the lower end of the inlet-limb. The level of the water inside the lower end of the outlet-limb had been depressed by the contained air to that of the water in the sealing-basin. Bubbles of air continued to escape while the water rose in the inlet-limb. As each bubble of air emerged there was a corresponding move upwards in the inlet-limb water-level. Consequently the water in the inlet-limb lagged only about a quarter of an inch behind the reservoir water-level, and it gradually attained the crest. The water then began to pour over the crest and passed down into the sealing-basin.

When the medium flow was being employed with 4-inch seal, siphon action was incomplete, and flow-through set in, with occasional irregular and

partial breaking.

When the slow inflow was used—that is to say, only sufficient to raise the water-level inside the throat to somewhere between the crest and just below the crown—all the inflow water drained away steadily through the siphon.

This run-through will occur in streams where the inflow is not sufficiently rapid to raise the reservoir-level quickly enough to produce siphonage. Owing to the small irregularities in stream-flow, the level in the siphon throat may fluctuate for long periods between the crest and just below the crown. During the whole of this time the water is running to waste in so far as siphon action, the required anti-mosquito measure, is concerned. Since no siphon action is set up, there is no lowering of the water-level in the reservoir.

The provision of a shallow water-seal, \(\frac{1}{4} \) inch, did not improve the per-

formance of the model, either with the medium or the slow inflow.

These experiments with medium and slow inflow were repeated, testing deeper water-seals, e.g., immersing the outlet-limb first to one inch and then to two inches. Corresponding results as regards water-levels were obtained. The lag in rise of the inlet-limb water-level increased with greater depth of seal. The escape of bubbles of air from the outlet was delayed in proportion as the seal was increased, owing to the greater depth of water through which the air had to be forced.

The results obtained from the use of three different depths of water-seal with medium and slow inflow are set out in Table I.

TABLE I
Showing the results obtained with different depths of seal

	Reservoir water-level at which bubbles of air	Reservoir-level when water in siphon flows	Siphon action		
	•	over crest	Medium flow	Slow flow	
1	About 3 above inlet About 2 above inlet	About 1 above crest	Abortive Abortive	None None	
2	About 4½ above inlet	About 2 above crest	Abortive	None	

It is seen from the above table that the use of seals of 1 and 2 inches yielded neither better nor worse siphonage results than the use of the 4-inch seal.

The provision of a basin water-seal of varying depths had not improved the performance of the model; on the contrary, it had rendered the siphon entirely useless, since even the medium flow, which had acted with moderate success when the model had the outlet open, was now a complete failure as a means of producing repeated siphon action.

In order to obtain the advantage of retention of water in the reservoir, followed by a sudden discharge through the siphon, during periods of medium and slow inflow, many methods have been adopted and a variety of apparatus designed. A preliminary measure recommended is the establishment of a water-seal on the outlet-limb; we have seen, however, that a water-seal alone is not an advantage in the case of the model, but quite the reverse. Another measure required is a means of breaking the siphon action with certainty; and a third is a mechanism for priming or starting the siphon. We may deal first with some of the different methods of water-sealing, but it will be observed that many of those described act also as agencies in priming.

Various methods of water-sealing. A basin water-seal such as that already mentioned has the effect of preventing air from entering the outlet-limb, but it also hinders air and water from escaping. In some siphons a sealing-basin is not employed. One method of providing a water-seal, inside the outlet-limb itself, depends upon a sheet of falling water or 'nappe,' which acts as a water diaphragm; the nappe prevents air from entering through the outlet-limb; it does not hinder the escape of air or of water from the outlet; on the contrary, it greatly assists this.

Speaking generally, any means by which a sheet of water can be made to extend across the whole internal area of the lower limb may be used; Naylor (1935) gives a review of the methods employed. The simplest device is the provision of a downward bend in the outlet-limb, in cases where this is sufficiently sloping, with the result that a sheet of water is shot across the lumen

(fig. 2, A). In long and very sloping lower limbs, more than one bend may be provided (fig. 2, B).

A second method is to make a step on the back wall of the lower limb, off which the water is shot across the tube (fig. 3, A).

Heyn has invented a flexible metal tongue which, being fixed just below the crest, continually directs the overflow at the crest across the outlet-limb of the

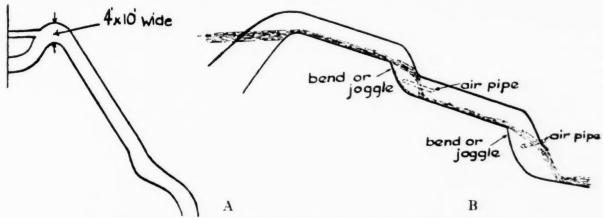


Fig. 2. A.—Bend in lower limb; B.—Long sloping siphon.

siphon; this acts as a seal until priming occurs (fig. 3, B). During the actual discharge, the weight of the water presses the steel tongue down against the back wall so as to allow unobstructed flow. The introduction of an internal mechanical device of this kind may, however, cause difficulty: it may go out of order, and it is inaccessible for repair, especially in small siphons.

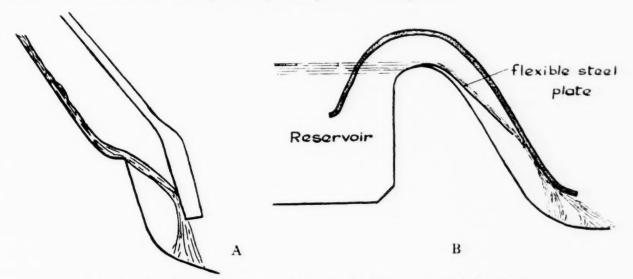


Fig. 3. A.—Priming step; B.—Heyn's flexible tongue priming device.

Nappe formation can also be obtained by the 'priming weir' (fig. 4, A). In this device, a weir is continued at a crest-level from the crest along the sides and across the back of the siphon, so that, when overflow commences, the falling nappes completely seal off the crown of the siphon. The air in it is gradually carried away by the falling water until priming ensues.

Yet another method of providing a nappe water-seal is the employment of a miniature internal siphon (fig. 4, B). In the throat of the main siphon a small auxiliary 'baby' siphon is built, having its inlet just below the crest. This siphon will flow full about the time the water reaches the crest-level of the main siphon. The sheet of water issuing from the baby siphon is directed so as to shoot obliquely across the lower limb of the main siphon, thus sealing it and preventing air from entering from below. The air which is then enclosed in the bend above the nappe is gradually carried away by the surface of the falling water until priming takes place.

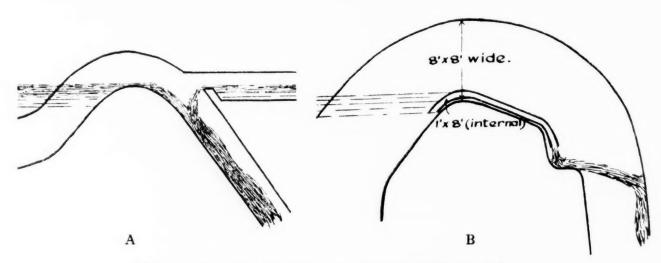


Fig. 4. A.—Priming weir; B.—Internal auxiliary siphon.

The auxiliary siphon is looked upon as having several drawbacks. Its presence acts as an obstruction to flow in the main siphon, and this is considered to be one very serious disadvantage. Other drawbacks are the expense of installing it, the difficulty of construction, a liability to choke, and the danger of fracture from vibration.

In cases where these nappe devices are used, a water-seal at the lower end of the outlet-limb is not generally employed, but in some siphons both a sealing-basin and one of the nappe-formation methods are employed together, in order to produce an efficient seal (fig. 5). These particular methods of sealing are employed for siphons of large size, and it has not been possible to obtain evidence of their use so far in anti-mosquito work.

Breaking devices. The second measure required with both the medium and the slow inflows is the provision of a means of breaking the siphon action. In order to make certain of complete cessation of the siphonage, it is essential in large siphons, such as those used in reservoirs, to have a separate air-vent for the purpose. This is rendered necessary because where much wave action is set up on the surface of the reservoir, owing either to the disturbance caused by the inflow or to wind, the admission of air to the siphon-inlet when the water-level falls may be irregular, thus causing partial or intermittent breaking.

A good deal of space in Naylor's treatise is given to a description of the methods by which siphon action may be broken, and some reference to these may be made, bearing in mind the fundamental differences in function that exist between the siphon which is used as a spillway and the type of siphon hitherto employed in anti-mosquito work.

Laggan dam represents the first instance in Great Britain of the embodiment of a large siphon spillway in the design. Naylor gives a full description of the construction of the siphons used and of their working. There are six siphons. Some idea of the size of the individual siphons installed can be obtained from the following facts. At the throat, which is rectangular in cross-section, each siphon is 6 feet 10 inches wide and 3 feet deep. The outlets of the siphons are 4 feet in diameter, and the length of the lower limbs is about 60 feet, measured from the crest; these are high head siphons.

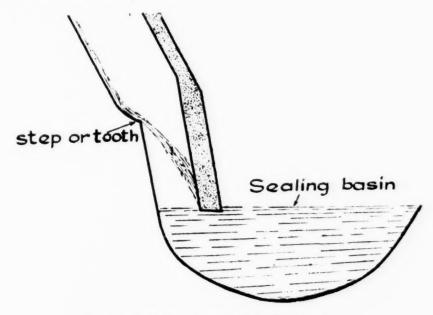


Fig. 5. Priming step and sealing-basin.

One classification of siphons is according to head: high, medium and low. Davies (1932-33) has suggested arbitrary figures: thus a high head siphon is over 20 feet, a medium from 10 to 20 feet, and a low up to 10 feet. In order that siphons may run full, without violent pulsation of flow, it is laid down that the lower limb of a high head siphon must be made convergent, that of a medium head siphon uniform, and that of a low head siphon divergent. In antimosquito work, siphons will almost always be of the low type, that is, under 10 feet.

The inlet of a Laggan siphon is immersed 8 feet in order to avoid downdraw of water, the formation of air vortices, and the intermittent making and breaking by wave action which would result if the inlet-lip were near the surface. Owing to this submergence of the inlet it becomes necessary to provide a special means by which the siphon action shall be stopped as soon as the water in the reservoir

falls to the desired level. In order to ensure the siphon breaking, an air-vent communicating with the interior of the siphon-hood is incorporated. For two of the siphons simple air-inlet lips are used; these are sealed by the rising and unsealed by the falling water; for the others automatic mechanical air-valves are installed.

With a rising level of the reservoir, corresponding to the medium flow of the model tests, or any faster flow, priming would eventually take place even if the air-vent were not there at all. But what would then happen with the medium flow would be that the siphon would run full bore continuously until the water-level of the dam had fallen as far down as the opening of the inlet-limb of the siphon, which is to be avoided. One of the main functions of this hood air-vent, then, is to arrest the fall of the dam water-level, by acting as a siphon-breaking device.

In other large siphons a curved air-pipe leads from the crown of the siphon down to the water in the dam. As in the case of the hood-vent, the closure of the lower end of the pipe by the rising water permits priming to take place, but the chief function of this air-pipe is to break the siphon action as soon as the water has been lowered again to the level of the air-pipe inlet.

In a similar way the Morgan mechanical valve, described and figured for some of the Laggan dam siphons, is a device which permits priming, but its chief function is to break the siphon action, so as to prevent the dam-level falling to the inlet-opening, which would be excessively low. The valve is caused to close by the rise of the reservoir-water; when the siphon has acted sufficiently the valve is caused to open by the fall of the water in the reservoir. Air can then pass through it and break the siphon action.

With these devices, as the reservoir water-level rises, the level of the water inside the siphon inlet-limb also rises, so that priming can occur very soon after the air-lip or mechanical valves have been closed. It is stated that the mechanical valves are so effective that they break the siphon positively and quickly when the water-level has fallen only three inches below the level at which the siphon primed.

Bunau-Varilla (1930) describes the fittings which he found necessary for priming and breaking the small siphons used by him. These fittings were adopted for an inflow which was capable of raising the reservoir-level at the rate of 2 cm. per minute. He found later that, by arranging the capacity of the siphon chamber so that the inflow caused a rise of level of at least 4 cm. per minute, special fittings for priming could be eliminated.

We saw, in the case of the model, that priming always occurred when a medium flow was used, but that regular siphon action would continue only if complete breaking could be effected.

Bunau-Varilla similarly found that he could not dispense with a special device for breaking the siphon action. He provided a tube to conduct the atmospheric air through the crown of the siphon; the lower end of the tube

reached down to the level in the reservoir at which it was desired to break the siphon action. When the falling water had reached this point it actuated a mechanism which suddenly lifted the end of the tube out of the water so as to let air enter. The apparatus designed for this purpose by Bunau-Varilla was a 'water-balance,' which he describes. The water-balance consists of two flat metal boxes joined together, the short side of one to the long side of the other. One of the two boxes is 4/5 full, the other empty and pierced with holes above and below in the two short sides. An axis of rotation is placed under the junction

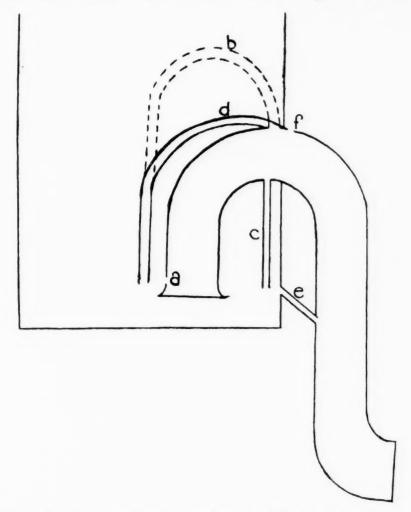


Fig. 6. Diagram showing various breaking arrangements.

of the two boxes. In air the balance falls to the side of the partially filled box, but when the instrument is immersed in water the opposite result is obtained. The breaking-tube is continued in a piece of rubber tubing, and the end of this is attached to the empty box; as this goes up when the water-level is sufficiently low, it will raise the tube into the air and cause complete and perfect breakage of the siphon.

The following methods of breaking were employed with the water-sealed model. A plain circular vent was made by drilling a hole in the inlet-limb of the siphon about an inch above the level of the inlet (fig. 6, a). This gave

irregular results, with only partial breaking, and was no better—in fact rather worse—than relying on the inlet itself to act as an air-inlet and breaker.

In order to provide an inlet to the crown in the glass model, a hole of suitable size was drilled, and a piece of glass tubing about 1 inch in length and inch in internal diameter was fixed in position. On this a piece of thin-walled rubber tubing of the same internal bore was fitted and carried over the wall of the reservoir and down into the water (fig. 6, b). It was found that this thin tube. as well as a thick-walled rubber tube subsequently tested, did not give good results, so curved and straight glass tubes with rubber junction-pieces were The lower end of the air-tube was placed half an inch above the level of the siphon-inlet. This position was selected as a result of experiment. the action of breaking being unsatisfactory when the air-tube inlet was lower than With the breaking air-tube so arranged, the water-sealed siphon acted automatically through almost the whole range of the medium flow. water rose in the inlet-limb of the siphon it was rising also in the air-tube, and both these water-levels lagged equally behind that of the reservoir. As soon as the throat of the siphon began to run full, the water in the air-tube was sucked over the bend, so that now the air-tube was acting as a subsidiary siphon. It went on doing so till the reservoir water-level fell to the inlet of the air-tube. when air entered to break the siphon action.

It was found that when the medium flow was increased in rate till it approximated to 'fast' flow, the breaking was apt to fail or to be incomplete. The result was that flow-through set in, and this might continue even when the water-level in the reservoir had fallen below the open end of the air-tube. When this occurred, it was observed that the air-tube contained a column of water which could not descend by gravity, owing to the suction exerted by the partial flow through the siphon. At the same time the suction was not sufficiently strong to pull the water in the air-tube over the top of the bend in it. The column of water in the air-tube therefore oscillated up and down, acting as a permanent water-seal in the tube, so that no breaking could occur.

In view of the fact that the air-tube acted as a subsidiary siphon and was full of water while the siphon itself was acting, it seemed that the curve in the air-tube over from the crown was too high. The result was that, by the time the reservoir-level had fallen to near the air-inlet and the siphon action was weakening, less suction was being exerted, just at the time when it was most required in order to draw in the air for breaking.

An alternative arrangement of air-inlet was tried, on a lead-pipe model of $1\frac{1}{2}$ inches in diameter, on which it was easier to adapt fittings. An air-tube was dropped perpendicularly into the reservoir from an aperture at the bottom of the siphon bend, so that the air might enter at the crest (fig. 6, c). It was thought that the short straight tube would clear itself of water more readily. However, the breaking was not satisfactory, owing to the air-tube being filled by the water in the main siphon as it passed over the crest.

A compromise was made by leading the air-tube almost on the level from the uppermost part of the crown and turning it down into the reservoir (fig. 6,d). This got rid of the high curve, and when the medium flow was employed regular automatic action resulted.

Still better results were obtained by employing as a breaking-mechanism a short length of straight metal tube sloping slightly downwards through the wall of the reservoir to join the outlet-limb (fig. 6, e). In the first experiments with it, the tube had its opening from the reservoir one inch above the level of the inlet of the siphon, and it gave good results. It was found to give equally good results within a fair range of levels. The best flushing was obtained when the exit of e from the reservoir was not more than a quarter of an inch above the level of the siphon-inlet. When the water in the reservoir fell during the siphonage and had uncovered the end of the tube, it did not stop at this level, but continued to fall right down to the level of the inlet of the siphon. This complete fall did not occur with any of the other arrangements and shapes of air-breaking tube previously tried. It was found that this very simple form of air-tube situated low down gave more violent siphonage, as well as effective and complete breaking. There was, however, a considerable delay in the start of the siphon action; the escape of water from the reservoir through the small tube prevented a rapid attainment of the necessary head; the interval between break and break was increased.

Priming. A third measure recommended is to provide some means by which, when the water in the inlet-limb reaches to about crest-level, the air confined in the bend and the outlet-limb of the siphon can be evacuated. While this measure is not essential in order to produce siphonage with medium flow, it is so in the case of slow inflow. The effect of the release of air is to permit the head of water in the reservoir to thrust the water in the inlet-limb up to the crown and over the bend, a process which starts the siphon action. It is this filling of the throat of the siphon with water so that it runs full bore which is referred to as 'priming.'

In the experiments with the glass model and a basin water-seal, it was possible to see that, as soon as the water inside the inlet-limb began to rise towards the crest, it pushed before it the air contained in the two limbs, and that this air soon began to escape as bubbles at the outlet. When the water ran over the crest it carried air down with it. With the medium flow, sufficient air could be gradually removed in this way, and the siphon throat at last filled, that is to say, 'priming' occurred, and the siphon action was set up. With slow inflow, however, sufficient air could not thus be removed, so that no priming action resulted and the water ran through continually.

In large siphons, when the 'nappe' produced by a sheet of moving water acts as the water-seal, the falling water itself helps to induce priming. It does this by carrying down with it the air from the bend of the siphon, until finally the throat can run full bore with water.

When a basin water-seal is employed and the inflow is slow, special means for removal of air from the region of the throat must be used in order to prime the siphon. Many devices have been invented with the object of producing this result rapidly and certainly. As a means of demonstrating the basis of some

of these devices it is convenient to use the lead-pipe model.

A hole 1 cm. in diameter was drilled at the point just anterior to the highest point of the crown (fig. 6, f). The siphon with its air-tube was embedded as before in the front wall of the reservoir and the outlet-limb sealed with a waterseal of two inches. The predetermined slow inflow was employed. as the filling of the reservoir began, a hand was pressed over the aperture in the crown. When the reservoir-water had reached a level above the crown, water began to flow over the crest of the siphon. The hand was now quickly released, with the result that siphonage at once commenced, but it stopped almost immediately. The water-level in the reservoir fell, but only to the level of the In order that siphon action might go on to completion, it was necessary to replace the hand over the aperture. The timing of this operation had to be found by repeated experiment. One means was to count the seconds; another was to watch the fall of the reservoir-water and, when it had reached a certain level, to reapply the hand. The most reliable method found was to observe the first rise or bulge of the water in the sealing-basin; when this bulge reached its maximum height, the hand was instantly reapplied. By any of these methods of timing, the opening and closing of the crown air-hole could be done in such a way that siphonage was produced with certainty once the water in the siphon had reached crest-level.

Even with very slow inflow the water in the inlet-limb always reached the crest eventually, and once it had reached this level priming and siphonage always took place with the model if air-release and closure were performed.

Air-pump. Naylor points out that, if the outlet-limb of the siphon is submerged, an obvious and certain method of bringing about priming is to evacuate the enclosed air from the crown by means of an air-pump or ejector. This method was adopted in the earliest siphon regulator, where water overflowed, at crest-level, through a small pipe. A branch pipe was connected with the crown of the siphon so as to form an air-ejector. He states that the air-pump or ejector is the only device by means of which priming may be initiated at will, with the water-level below the crest. The air-pump can be applied to any shape of siphon, provided that there is, throughout the priming, a water-seal to the outlet-limb. One great disadvantage, however, is that, should anything happen to impair the proper functioning of the air-ejector, the dam would be endangered; the air-passage being small, choking by debris is by no means impossible.

Efforts to utilize this method with the model failed entirely when a really slow flow was employed, on account of air being sucked into the overflow-pipe; this kept partially priming and then breaking the siphon. Attempts to keep the air-ejector supplied with water from a separate small tank fitted with a siphon

leading to the air-ejector also failed. The conditions in anti-mosquito work are such that, as the stream dries, there may be insufficient water entering the siphon reservoir to ensure an adequate overflow to such an air-ejector. This source of failure to prime will also arise in the case of siphon spillways, but, if it does, no harm is done because the action of the siphon in such conditions of low water is not required.

There are several means by which air may be released from the crown. One is the use of one of the 'nappe' sealing-methods mentioned above, the fall of the water over the crest carrying the air down and out of the outlet-limb. This has limitations, as it requires a considerable height of siphon and a good flow of water over the crest, which would occur with a medium flow but not with a very slow flow. For anti-mosquito work it is necessary to devise methods which will be applicable when short siphons are used, and which will be effective when only a very slow inflow can be obtained. The action of the siphon may be most desirable just at such times as the inflow is particularly slow and the reservoir fills infrequently.

Another means is to provide an aperture in the crown, and by some method to imitate the timed opening-and-closing action carried out by the hand in the above experiments with the model; various valves have been employed for this purpose with more or less success.

In Malaya, siphons were seen in which an air-pipe leading from an aperture in the crown was used as an air-release. The arrangement of a series of small concrete reservoirs and siphons provided for the opening-and-closing action without the use of any moving mechanical parts.

Recently Macdonald (1939) has published an account of a design of siphon which has given good priming results and is easily manufactured. For evacuating the air contained in the throat of the siphon, Macdonald employs an internal ejector instead of an external one; the water which operates the ejector is derived not from the overflow at the top of the dam, but from the water rising in the inlet-limb itself (fig. 7). It provides an ingenious and simple solution of the priming problem. There is a similar small waste of water through the ejector, depending on the bore of the pipe used.

In Macdonald's design a small pipe with open ends reaches from the throat, at a level a little below the crown, down through the dam wall and curves out below the water-seal; it has a short open branch which leads into the inlet-limb just below the siphon-crest; alternatively this branch is continued, as shown in the figure. As the water rises in the inlet-limb the air displaced in front of it passes freely down the pipe without disturbing the water-seal. When the water in the inlet-limb reaches the level of the branch pipe, it flows down it; in doing so it sucks air out of the throat of the siphon, so that the water can rise over the crest, fill the throat, and start siphon action. The siphon is strongly made of reinforced concrete in three pieces, which can be transported and assembled locally.

Limitations to the use of a crown air-release in priming. The provision of a crown air-release mechanism is not of any assistance in promoting siphoning in an open-outlet siphon, whichever rate of flow is used. With the outlet sealed the results with the models were:

(a) Fast flow. The air-release is of no assistance, as the water keeps running through full bore, while the reservoir-water rises to overflow.

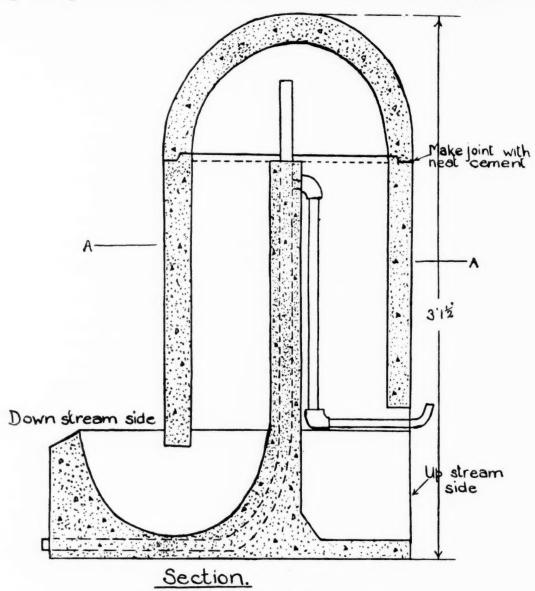


Fig. 7. Anti-malarial flushing siphon.

(b) Medium flow. Here the air-release is of value chiefly as a measure for preventing waste of water, since priming will take place in time without it, and regular automatic siphonage will result, if a proper means of breaking is provided.

(c) Slow flow. In this case alone is some form of air-outlet absolutely essential; the air from under the crown must be evacuated by some mechanism before the siphon can act.

A series of experiments was carried out, using the two model glass siphons of $\frac{1}{2}$ - and 1-inch internal diameter, and the lead one of $1\frac{1}{2}$ -inch diameter. The performance of these without and with a basin water-seal was tested, first as simple siphon tubes and then when they were supplied with various fittings. The three flows, fast, medium and slow, were employed with each arrangement of the siphon. The fast flow in every case resulted simply, as previously noted, in a continuous run-through of the water, while the reservoir-level rose to over-flow point. The results obtained with medium and slow inflow are given below in tabular form.

Table II
Showing the results, with medium and slow inflow, of different arrangements of the siphon

	Cial and annual and	Inflow			
	Siphon arrangement	Medium	Slow		
1.	Without water-seal* Without breaking device	Irregular siphon action; may not break properly. Much waste before each action	No siphon action; run-through all water to waste.		
2.	Without water-seal* With breaking device	REGULAR automatic siphon action. Much preliminary waste	No siphon action; run-through		
3.	With water-seal Without breaking device Without crown air-release	Abortive siphon action; then run-through	No siphon action; run-through		
1.	With water-seal With breaking device Without crown air-release	REGULAR automatic siphon action; some waste	No siphon action; run-through		
5.	With water-seal With breaking device With automatic crown air- release	REGULAR automatic siphon action; very little waste; crown air-release really superfluous, but may reduce waste slightly	REGULAR automatic siphor action; very little waste; crown air-release necessary for siphon action.		

^{*}In the absence of a water-seal, a crown air-release cannot be of service.

Remedies for the fast stream-flow. As previously stated, it is possible to make modifications which may render the fast inflow more serviceable for anti-mosquito work. The flood-water conditions which give rise to fast flow may in themselves be sufficient to eliminate anopheline-breeding, owing to the volume of water and the violence of its passage down the stream-bed, with effects somewhat comparable with those described above as likely to result from siphon discharge. In fact, this appears to be what occurs during the wet season in most tropical countries with definite heavy rains. At the commencement of the rainy season isolated downpours, succeeded by dry days, greatly increase the anopheline breeding-places by the formation of new pools beside the edge of the

stream. Similar types of breeding-place are apt to be left when, at the end of the wet season, the rain again becomes intermittent. But during the period of steady rains breeding is diminished in such pools and in the streams themselves, owing to the flood conditions. However, should it be considered desirable to try to take advantage of the periodic discharge of siphons even during floods there are several ways in which this may be done.

1. A larger siphon can be installed. If this operates adequately during the high-water period, under low-water conditions it will fail to act unless it is provided with the fittings necessary for producing siphon action during slow

inflow.

2. An additional siphon or siphons can be installed at the same level, arrangements being made that any of them can be left out of action during

normal conditions, while they all act during floods.

3. Probably the simplest remedy is to regulate the inflow from the stream to the siphon chamber, so that the medium flow previously described is obtained, giving automatic periodic discharge. In order to effect this, any excess of water in the stream is made to by-pass the siphon reservoir. A suitable arrangement is the building of an accessory reservoir of such construction that, when it is full, a pipe or outlet from it delivers a constant inflow to the siphon reservoir. Any excess of water above this requirement is allowed to spill over the front of the wall of the accessory dam directly into the stream. By this means the siphon reservoir is always supplied, even during floods, with an inflow at the medium or optimum rate.

If arrangements can be made to ensure the inflow to it being constant, and of the medium rate described, even a simple unsealed type of siphon can be employed with moderate success, although there will be a very considerable waste

of water each time before the siphon acts.

Rise-and-fall siphon. Another type of siphon has been developed which works on a different principle from any of those previously mentioned. In correspondence with Mr. Morgan, the inventor of the Morgan valve, I enquired whether any modification of his valve could be adapted for the purpose of siphons for anti-mosquito work. At the same time I stated that the difficulty was to discover some means by which preliminary loss of water, before siphon action commenced, could be avoided, so that full advantage could be taken of very small rates of flow in streams.

He suggested that, while modifications of his own valve would not meet the case, the effect required might be obtained by a siphon with one moving part. The siphon itself would float up till it reached a fixed stop; surrounding the siphon near the top a collar would be provided which, when the stop was reached, would fill with water, causing the siphon to sink rapidly. The downward thrust of the siphon would force the water over the crest, fill the throat, and start siphon action. The collar would then be emptied by a small siphon fixed in it. The idea was somewhat similar to that of an apparatus designed

and used by Williamson in Malaya. In this, a large empty drum with a hole in the top was employed. To the lower part of the drum a pipe consisting of bamboo led through the dam wall. A rubber connection permitted movement.

The first experiments with a wooden model of this rise-and-fall siphon were not a success. During experiments with a model made of tin I was able to make several modifications, as a result of which progress was possible. The first difficulty which had to be overcome was that the small siphon failed to employ the collar regularly. The use of the small siphon was therefore abandoned, and drainage of the collar was effected by a pipe leading down from the bottom of it to join the horizontal limb of the siphon. The collar itself was next

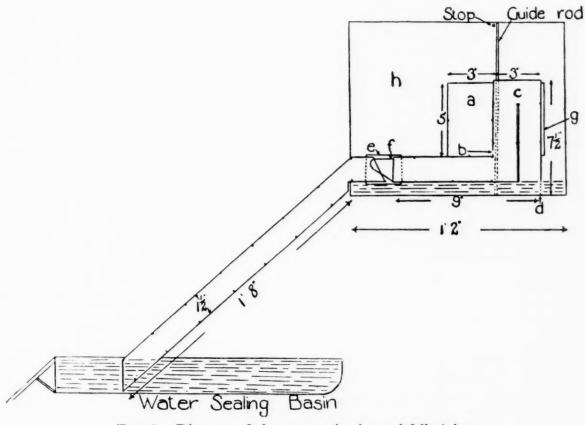


Fig. 8. Diagram of the automatic rise-and-fall siphon.

eliminated and was replaced by a simple box. Then it was found that this box (fig. 8, a), which acted first as float and then as sinker, was quite effective when fixed directly on the top of the horizontal limb. The next step was to make a small drainage aperture (fig. 8, b) near the bottom of this box, leading into the down limb, in place of the pipe to the horizontal limb of the siphon.

The tins employed for making the model were like kerosene-tins in miniature, soldered together end to end, the resulting pipe being of square section. Curves in the siphon itself were then eliminated, which made the construction much easier. Although, no doubt, the angular siphon (fig. 8, c) increases resistance to flow, the performance of the model is good. The only

loss of water which occurs when this apparatus is in action is a very small quantity which escapes from the horizontal arm when the siphon begins to float up on the rising water of the reservoir. This small residual quantity is derived mainly from the drainage out of the float-sinker into the descending siphon limb.

Guide-rods: two perpendicular rods are fixed to act as guides between which the siphon rises and falls.

In order to stop the siphon quite reaching the bottom of the reservoir, which would obstruct the flow into the siphon mouth, two curved pieces of wire are fixed to project about half an inch and give the siphon that amount of clearance from the floor as shown in the dotted line fig. 8, d.

The joint (fig. 8, e) in the model is made with rubber, but any flexible and waterproof material, such as waterproof canvas, could be employed. end-to-end adaptation of the pipes, the one being fixed, the other moving, and also to prevent the rubber from being caught between the ends, the moving part of the model is provided with two projecting curved wire guides (fig. 8, f), which serve both purposes. Since water is passing through the pipe only when it is lying in a horizontal position, a projecting tongue of metal, an extension of the upper wall of the moving part, passing into the fixed pipe, could also be provided without obstructing the flow.

By adjusting the size of a piece of metal attached to the siphon (fig. 8, g) and used as a weight, it is possible to reduce the buoyancy of the siphon as required, to permit it to sink immediately the water in the reservoir h commences to flow into the float-sinker box when the stop is reached.

With a water-seal of a quarter of an inch depth above the upper margin of the outlet-pipe, this model delivers automatically, from the slowest inflow obtainable, seven and a half gallons per minute.

Acknowledgements are due to Professor Naylor and Messrs. Edward Arnold, for permission to use several diagrams from 'Siphon Spillways' (figs. 1-5), some of which have been slightly modified; and also to Dr. Macdonald, for permission to use the diagram of his design of anti-malarial siphon (fig. 7); at the same time I wish to thank Professor Scholes, of the Department of Mechanical Engineering, University of Liverpool, and Mr. Morgan, for their helpful advice.

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EXPERIMENTS WITH ISOLATED MALARIA PARASITES (PLASMODIUM KNOWLESI) FREE FROM RED CELLS

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In a previous communication (Christophers and Fulton, 1938) we have described certain experiments with malaria parasites (*P. knowlesi*) in bulk. In these experiments we were dealing with parasites still included in the host cell. Such efforts as were made to obtain material free from the red cells by laking with water or weak salt solutions were unsatisfactory, in that the parasites so obtained were to a considerable degree fragmented or otherwise altered. In the present experiments saponin was used with satisfactory results to bring about lysis of the host cell. Not only do preparations of saponized material, fresh or stained, show the parasites in a condition apparently little altered, but such material, even after washing several times, still shows some oxygen uptake and is infective when inoculated.

Saponin-haemolysis has the additional advantage over haemolysis by water that, whereas in the latter the haemoglobin passes out, leaving the stroma still present, in saponin-haemolysis it is lysis of the stroma which brings about liberation of the haemoglobin, so that the red cell completely disappears.

MATERIAL AND METHODS

The following procedure was adopted in obtaining our material. Parasitized cells were isolated as described in our previous paper, and the volume of material was determined. It was then suspended in an equal volume of physiological saline, and a volume of 0.2 per cent. saponin in saline equal to that of the suspension was added. After thorough mixing, the preparation was incubated at 37° C. for half an hour. Experiments had shown that these were the most suitable conditions for obtaining complete haemolysis when serum was absent. If, in place of saline, a suspension was made in monkey serum, the addition of 0.6 per cent. saponin solution, in place of the 0.2 per cent., was found to be necessary to bring about complete haemolysis.

^{*} Work carried out under the Medical Research Council.

After incubation and verification that no red cells were present, the material was centrifuged and the supernatant fluid, charged with haemoglobin, removed. The deposit, now of a greyish-black colour, in contrast with the chocolate-brown colour of the original unsaponized material, was resuspended and washed in three further changes of saline or Ringer, the last change remaining free from any tint of haemoglobin. Unless otherwise stated, such material was used in our experiments.

Examination of the fresh material showed parasites to be entirely free from red cell or red-cell stroma. Such parasites were rounded, but with perfect contour and with no evidence of fragmentation (Plate III, fig. 4). Films made from the deposit and stained with Leishman's stain showed the parasites free from red cell and with the chromatin staining in an apparently normal manner. In thicker parts of the film parasites tended to be rounded up as compared with those seen in the usual blood preparation, but on thinner portions they were more spread out and did not differ greatly in appearance from similar parasites seen in their host cell (Plate III, fig. 5).

In those cases in which serum was used throughout in place of physiological saline (i.e., all processes including washing carried out in serum, with the necessary increased amount of saponin), similar results were obtained, except that there was a tendency for fine floccules to form, making the suspension less suitable for most purposes.

Apart from the preparation of material as described, in which the parasites were of large size (usually three-quarters to full grown), the saponin method has also been used with blood heavily parasitized with ring forms. These young stages of the parasite do not lighten the red cell sufficiently to allow of separation by centrifuging, as in the case of older stages. But, by treating the deposit of red cells separated from serum in the centrifuge, this stage of the parasite can also be prepared in mass, though the volume is very small.

RESULT OF INOCULATION INTO ANIMALS OF WASHED SAPONIZED MATERIAL

Although we have not ourselves inoculated animals with material obtained after haemolysis by water or hypotonic salt solution, Ciuca, Ballif and Chelarescu-Viéru (1934) found that parasitized blood haemolysed by sterile distilled water did not give rise to infection, nor did it influence the course of a subsequent inoculation with infected blood. Also Sinton and Mulligan (private communication) have inoculated haemolysed *knowlesi* parasites into *Macacus rhesus* without effect, having used water as haemolytic agent.

The following results were obtained by us with saponized parasite material prepared as described and inoculated subcutaneously immediately after washing, the animals used being M. rhesus.

TABLE

Monkey source	Nature of preparation	Form of parasites	Monkey inoculated	Amount inoculated c.cm.	Result	Remarks
149	Saponized parasite substance	Large forms	160	0.5	Positive 14th day	_
156	"	,,	164 166 167	0·2 0·2 0·2	Positive 11th day Positive 10th day Negative	
167	>>	,,	171* 172*	0·2 0·2	"	Inoculation some hours after pre paration
158	Saponized red cell deposit	Predominantly ring forms	159		Positive 11th day	

In all cases but one, where the material was inoculated without delay after washing, a positive result was obtained, but generally the incubation-period was lengthened. The course of the infections was normal. Examination of stained slides, taken at the time of inoculation, showed that removal of the red cell was complete. In regard to the preparation from no. 167, which had been left compacted some hours after centrifuging, it may be noted that normal parasite substance left overnight in similar circumstances had previously failed to cause infection. Both large forms and ring forms after saponin treatment have given positive results, but other stages in minimal numbers may have been present. Also, in the case of the large forms, segmenting parasites with merozoites cannot be excluded.

DETERMINATION OF THE DRY WEIGHT OF PARASITE SUBSTANCE

In the paper quoted, we gave some determinations of the dry weight of parasite substance freed from red cells by haemolysis with water or hypotonic salt. The results were not considered final, since the effect of the haemolytic agents used on the parasite was not known. A revised determination, using a large quantity of the saponized material, was carried out with the following result: 14.6 c.cm. of standard parasite substance gave, after saponin treatment

^{*}Note added June 23rd, 1939. The two animals nos. 171 and 172 which, as shown in the table, remained negative after inoculation with saponized material left some hours compacted without glucose, were reinoculated, along with no. 174, with similar material in the fresh condition. Parasites were detected in the blood of nos. 171 and 172 on the 10th and 13th day respectively. The infection followed the normal fatal course. No. 174, however, failed to show infection after three weeks.

and washing, 11.3 c.cm. of saponized deposit free from red cells. The dried weight of this material was 0.6495 gm., giving as the dry weight of 1.0 c.cm. saponized deposit 0.0575 gm. (as against 0.041 gm. previously given after use of water or hypotonic salt). If the proportion of saponized deposit to original parasite substance be, as found in this case, 77.4 per cent., the dry weight of actual parasite in parasite substance, as originally obtained by centrifuging, would be 0.045 gm. per 1.0 c.cm. of the compacted deposit (as against 0.03 gm. previously suggested). This figure is, however, bound to vary with the exact state of development of the parasite, which conditions the amount of red cell present in any given preparation.

RESPIRATION EXPERIMENTS WITH ADDITION OF SAPONIN DURING DETERMINATION OF OXYGEN UPTAKE

In order to ascertain the effect of saponin as an inhibitor of oxygen uptake on the lines previously described for a number of compounds, experiment 1 was carried out, in which 1.0 c.cm. of a solution of saponin in Ringer of the concentrations noted was added during the course of the experiment to the usual mixture of 3.0 c.cm. containing 1.0 c.cm. of 1:4 parasite suspension in serum, and an equal amount of Ringer and of M/15 phosphate buffer pH 7.4, the controls being similar preparations in which 1.0 c.cm. of Ringer without saponin was added from the side arm. The figures indicate O_2 uptake in mm.³

EXPERIMENT 1

T: :	O ₂ uptake in mm. ³					
Time in minutes	Control flasks (1.0 c.cm. Ringer added)	1.0 c.cm. of 0.1 per cent. saponin added	1.0 c.cm. of 1.0 per cent saponin added			
30	49.8	46.6	46.9			
50	71.5	$76 \cdot 2$	73.9			
65	88.1	94.0	96.9			
175	157.4	173.7	163.8			
275	188.3	$207 \cdot 2$	177.0			

Addition of side-arm contents made at end of 30 minutes.

From slides made of the flask-contents at the end of the experiment, it was seen that most of the red cells were haemolysed in both concentrations of saponin. Contrary to expectation, therefore, saponin in this experiment, though present in amount sufficient to haemolyse most of the host cells, did not cause reduction in O₂ uptake. A somewhat similar result, but in this case with some reduction, was obtained when using a slightly higher saponin concentration as shown in experiment 3, column 2.

As it was uncertain in the presence of serum to what degree haemolysis was complete at any given time, observations were made with mixtures in which the serum used in making up the suspension was replaced by Ringer. It was known from numerous trials that with a final concentration of 0.1 per cent. saponin in such a mixture parasite substance was always completely freed from red cell at the end of 30 minutes or less. It was also known that parasite substance suspended in Ringer and buffer without admixture of any added serum would show marked O₂ uptake, provided that glucose was present.* The following modified experiment was therefore carried out. In each of 6 flasks were placed 3.0 c.cm. of a mixture of equal parts 1:4 suspension of parasite substance in Ringer, 0.6 per cent. glucose in Ringer, and buffer, 1.0 c.cm. of Ringer and 1.0 c.cm. of 0.6 per cent. saponin in Ringer being present respectively in the side arms of the three control and three experimental flasks. The concentration of saponin was here more than sufficient to bring about complete haemolysis of all red-cell material in 30 minutes or less, and at the end of the experiment complete haemolysis was verified. Experiment 2 gives the mean results obtained. The experiment shows that haemolysis of the host cell brought about by saponin causes only a moderate decrease in O₂ uptake, and not complete cessation, as might have been supposed.

EXPERIMENT 2

15-minute intervals	O ₂ uptake in mm. ³ in successive 15-minute intervals		
	Control flasks	Experimental flasks	
1	58.1	54.9	
2	$50 \cdot 5$	46.5	
3	$35 \cdot 2$	$29 \cdot 9$	
4	$23 \cdot 2$	15.4	
5	14.3	12.0	

The contents of the side arms were added at the end of the first 15-minute interval.

RESPIRATION EXPERIMENTS WITH WASHED SAPONIZED MATERIAL

In the experiments just described the haemoglobin liberated from the haemolysed cells was still present. In the following experiments saponized deposit was used, entirely free from red cells or haemoglobin, and washed three times in saline or Ringer.

^{*} Observations on the utilization of glucose by the parasites will form the subject of a further communication by one of us (J. D. F.).

The results given in experiment 3, column 1 (controls), relate to 1.0 c.cm. of 1:4 suspension of parasite substance in serum and 1.0 c.cm. of buffer, with 1.0 c.cm. of Ringer in the side arm. The results in column 2, which have been previously referred to, relate to a similar preparation, but the Ringer in the side arm contained 1.0 per cent. saponin. In column 3 the results are those

EXPERIMENT 3

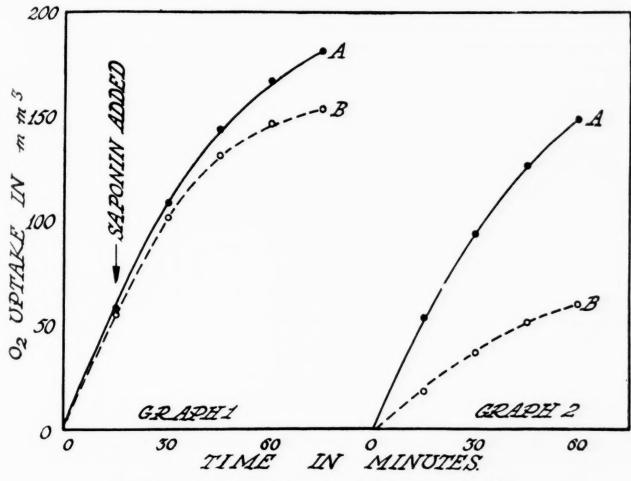
15-minute	O_2 u	iptake in mm. ³ in succe	essive 15-minute intervals
intervals	Column 1 (controls)	Column 2 (saponin added)	Column 3 (previously saponized material)
1	34.6	37.5	16.5
2	32.3	$32 \cdot 2$	11.6
3	27.7	25.0	9.8
4	25.4	24.9	10.1
5	16.5	18.1	7.9
6	16.4	10.9	3.5
7	16.3	6.6	5.2
8	12.4	$6 \cdot 2$	4.3

for 1.0 c.cm. of 1:4 washed saponized deposit in serum and 1.0 c.cm. of buffer, along with 1.0 c.cm. of Ringer in the side arm. Two flasks were used in each experiment, and the material from the side arms was added after 60 minutes' shaking at 37° .

EXPERIMENT 4

15-minute	O ₂ uptake in mm. ³ in successive 15-minute intervals		
intervais	Normal preparation	Washed saponized material	
1	53.4	17.8	
2	40.1	19.4	
3	$32 \cdot 1$	13.1	
4	22.7	9.8	

In experiment 4 a comparison was made of O_2 uptake by $3\cdot 0$ c.cm. of a normal parasite substance preparation (1:4 parasite suspension in serum, $0\cdot 6$ glucose in Ringer and buffer) with that of saponized material from the same source washed three times in $0\cdot 2$ glucose-Ringer and put up in a similar manner. The material was the same as that employed in experiment 2.



Graph 1. A.—Control flasks. B.—Flasks with saponin added. Graph 2. A.—Control flasks. B.—Washed saponized material.

The results of experiments 2 and 4 are illustrated in the accompanying graphs.

In experiment 5 are given oxygen-uptake measurements, using washed saponized material in the presence and absence of 0.2 per cent. glucose. The

EXPERIMENT 5

15-minute	O ₂ uptake in mm. ³ in successive 15-minute intervals		
intervals	Glucose absent	Glucose present	
1	8.2	17.5	
2	6.0	11.7	
3	$6 \cdot 4$	9.9	
4	$5 \cdot 2$	9.1	
5	2.7	2.7	
6	0.0	4.9	

serum used in the previous experiment was replaced by Ringer, i.e., the contents of each flask consisted of 1.0 c.cm. of 1:4 suspension of washed saponized deposit in Ringer, 1.0 c.cm. of buffer and 1.0 c.cm. of Ringer (with and without glucose).

It would appear, therefore, that, even after the very drastic treatment entailed in saponizing the host cell and in washing in saline or Ringer, and in the entire absence of serum and of haemoglobin, a small oxygen uptake still took place. The uptake was greater in the presence of added glucose.

CONCLUSIONS

The use of saponin enables parasites to be isolated in mass in absence of either red cell or haemoglobin, in a condition much less altered than is the case when water or hypotonic salt is used for the same purpose. The parasites so obtained show little or no fragmentation and stain normally. Some parasites must remain viable, as the material has proved infective on inoculation, but the rather long incubation-period suggests that actually infective material was either small in amount or at a disadvantage in establishing itself.

Addition of saponin from the side arm during the experiment, sufficient completely to haemolyse the host cells, reduced but did not bring about cessation of O₂ uptake. This was the case whether the original suspension was made in serum or in Ringer only.

Washed saponized material free from red cell or haemoglobin also showed a small oxygen uptake. In an experiment in which serum was omitted the O₂ uptake was greater in the presence of added glucose. Further experimentation is necessary before assessing these results.

Ability to obtain isolated parasites in bulk in absence of red cell and haemoglobin, in a better state of preservation than has hitherto been found possible, offers increased facilities for physical and chemical studies of the parasites. The accompanying photographs will serve to indicate the possibilities of the method.

ACKNOWLEDGEMENTS.—We wish to record our thanks to Mr. George Smith, M.Sc., of the Biochemistry Department of the London School of Hygiene and Tropical Medicine, to whom we are indebted for the microphotographs which accompany this paper.

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EXPLANATION OF PLATE III

- Fig. 1. Heavily parasitized blood of *M. rhesus*, as used in preparation of parasite substance; stained preparation.
- Fig. 2. Parasite substance, showing parasites still surrounded by the host cell; stained preparation.
- Fig. 3. Parasite substance as in fig. 2; fresh unstained preparation.
- Fig. 4. Parasite substance as prepared after saponin treatment, showing parasites in mass, free from the host cell; fresh unstained preparation.
- Fig. 5. Parasite substance as in fig. 4; stained preparation.
- Fig. 6. Ring-stage parasite material as prepared by saponizing red cells heavily infected with ring forms; stained preparation.

Magnification in all cases 500.

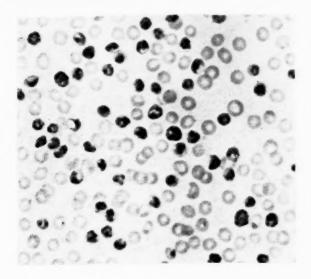
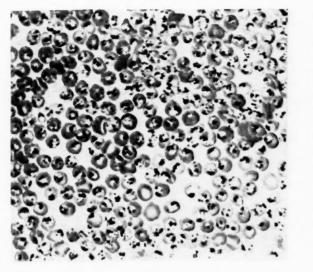


Fig. 1

Fig. 2



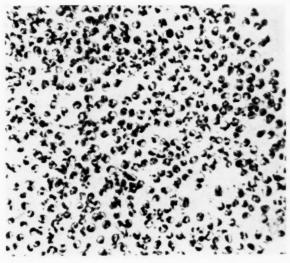
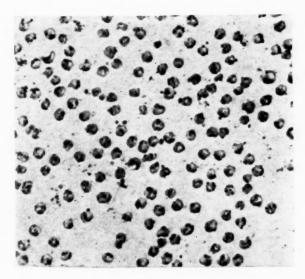


Fig. 3

Fig. 4



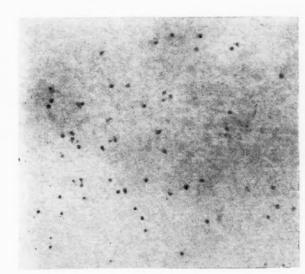
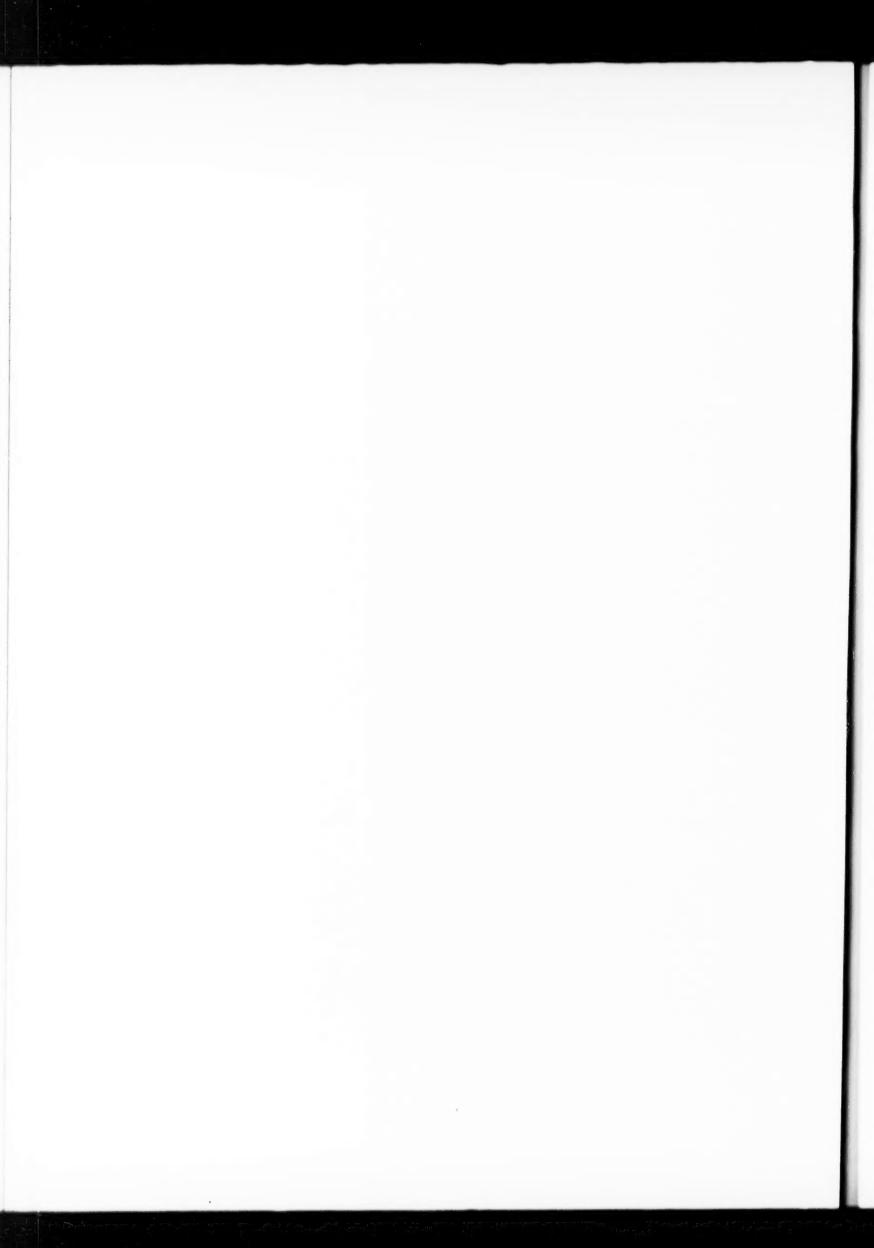


Fig. 5

Fig. 6

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PROBLEMS AND PROGRESS IN CHEMOTHERAPY*

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(Received for publication March 10th, 1939)

During the last few decades considerable success has been attained in the therapy of infectious diseases. And, since this is a subject of importance to everyone, more or less sensational revelations are sometimes expected of one who works in the field of 'chemotherapy' or who reports on the results of such investigations. But I do not intend to deliver sensational papers. My lectures will only summarize—as objectively as possible, as is customary in science—the results obtained in this branch of drug investigation, which is based on the same fundamentals as any other branch of medical research, and is closely related to the development of exact science and to all other fields of investigation in biology and medicine.

Chemotherapy is the causal therapy of infectious diseases by chemical compounds, which may be produced by synthesis, or be of half-synthetic origin, or be isolated from natural drugs. I myself think that it would be wiser to speak of 'drug therapy of infectious diseases,' and to drop the expression 'chemotherapy'; but the shortness and convenience of the expression 'chemotherapy' have led to its general use. The expression 'infectious diseases' must be understood in its widest sense, i.e., diseases caused by infection of human beings or animals with viruses, bacteria, protozoa or worms. Chemotherapy is simply a part of pharmacology, differing only from organic pharmacology in that in chemotherapy we have to deal with somewhat more complicated conditions.

While pharmacology investigates the influence of a substance upon the organs or cell-function of one organism, in infectious diseases we have to deal with two organisms, namely, with that of the host and with that of the parasite. Drugs tested for therapeutic activity must of necessity act simultaneously upon both. There is, however, no difference in principle, and we shall see later how closely these relations are interwoven. It is consequently surprising that in the last few years the question has been seriously discussed whether the continuance of pharmacology as a separate department in teaching and as a special branch of medical investigation is justifiable. It has been suggested that pharmacology could conveniently be split up into a series of special

^{*}Three special University lectures in pharmacy, given, under the auspices of the University of London, at the Wellcome Research Institution, London, on March 7th, 8th and 9th, 1939.

branches, and that chemotherapy should be treated as an independent subject. Chemotherapy is an excellent example for illustrating how deplorable an effect such specialization would have.

The pharmacologist, at the end of the last century and at the beginning of the present century, was essentially occupied in analysing and describing the effects of drugs. In recent years, however, a great change has taken place. The abstract-theoretical, the purely analytical, and the descriptive methods of investigations have built up for us, after much difficult and detailed work. the fundamentals upon which we continue our work of creating new drugs and of rendering possible their practical application. To-day, moreover, the pharmacologist no longer confines himself to ascertaining and registering the alterations of a cell, or organic function, produced by drugs or chemical compounds; his efforts lie rather in finding out the ultimate method of the action of drugs. He investigates the question of drug absorption, the distribution of drugs in the organism, their behaviour and their action on metabolism, their activation or inactivation, and their excretion. In general, it has been recognized that alterations in the functions of cells or organs are due to the action of the substance upon the cell-metabolism. There thus arises the possibility of drugs acting in a causal-therapeutic sense, that is to say, of their restoring the physiological activity of disturbed organ- or cell-functions by regulating the cellmetabolism.

Let us consider these questions more closely with the help of a few examples, so that we may be in a position to approach another problem which is of great importance in chemotherapy, namely, the question of the relationship between chemical constitution and effect.

Among the hypnotics, the derivatives of barbituric acid have gained special practical importance. In the case of these derivatives, it has been proved that the compounds with short alcylic side-chains are of relatively lower hypnotic action than compounds with increasing length of the side-chains. The displacement of the distribution-quotient lipoid to water in favour of the solubility in the lipoid varies in a parallel manner. If no other properties were of importance for the effect, those derivatives of barbituric acid which have the longest alcylic side-chains would be of optimal effect. That, however, is not so, for the simple reason that compounds with very long side-chains are not absorbed after oral administration, and consequently must of necessity be without effect. Thus we see how one alteration of the chemical constitution causes the modification of several physical properties, which in their summation are of decisive influence on the pharmacological behaviour of the compounds.

Let us now consider the case in which the compounds to be compared are well absorbed and are distributed in a favourable way throughout the body. Under these conditions the behaviour of the compounds in metabolism has a decisive influence on their effect. In the case of barbiton—the diethylbarbituric acid—for instance, only about 25 per cent. is decomposed in the body; about

75 per cent. of the compound, which is relatively stable in metabolism, is excreted in the urine, and this excretion extends over a relatively long period. It will at once be clear that prolonged effects are obtained with barbiton, that considerable after-effects may ensue, and that repeated doses readily produce cumulative effects. More labile derivatives of barbituric acid behave quite differently in metabolism. In phanodorm and evipan there are double junctions, to which oxygen is easily added. Hence, they are very quickly inactivated, and undergo such rapid and far-reaching destruction that only a small percentage at most is excreted in unaltered condition. In hypnotics it is the rapidity of inactivation which determines whether the compound is practically free from after-effects and of value only as a soporific. The same holds true of the derivatives of barbituric acid which contain bromide-substituted, unsaturated side-chains. Here, inactivation occurs by substitution of the bromide atoms by hydroxyl groups, consequent dislocation and eventual oxidation (cf. Weese, 1936). We encounter these relations between the behaviour of a drug in metabolism and its effect in many other branches of pharmacology. May I remind you of the sympathico-mimetic substances, adrenaline, sympathol, ephedrine, or the glycosides which affect the heart and the circulation, strophanthin and digitalis. The more unstable they are, the shorter is the period during which they are effective.

Furthermore, the effect is also dependent upon the special physiological properties of the organ upon which the drug acts. The absorbed substances are carried by the circulation to all parts of the body, and penetrate—although with varying speed—into the cells or cell-systems, where eventually they are stored. But that is not an absolutely elective process, because we find the hypnotics, for instance, in practically all organs of the body, and after prolonged action of large quantities the difference in concentration in the individual organs is by no means considerable (Keeser, 1935; Vogt, 1935; Drohocki, 1939). But the effect of the substances upon the easily recognizable, characteristic function of the nerve-cells is clearly apparent. Undoubtedly, an hypnotic or narcotic effect is exerted upon other cells or cell-systems, and is manifested by diminished function. In the case of substances which apparently act so specifically we have thus always to deal with effect and collateral effect, which are partly conditioned by the properties of the substances and partly by the properties of the cells on which they act.

The investigator in chemotherapy strives after somewhat different aims. He is not concerned with restoring diseased cells to their normal function or with bringing about a reversible alteration of the cell-functions. He aims at reaching the parasite-cells and at throwing their function so irreversibly out of order that the parasite dies but the host remains as unharmed as possible. The achievement of this optimal aim is still hypothetical, for in practice no one has yet succeeded in attacking the parasite only and leaving the host absolutely undisturbed. It is obvious that the compounds which are to kill the parasite

must be taken up into the body of the host, that is to say, they must be absorbed whether through enteral or parenteral administration. All the tissues of the host necessarily come into the closest contact with the drugs, and are inevitably influenced by them in one way or another. It is important, therefore, that their influence upon the tissues of the host shall be less than their action on the parasite. Furthermore, the action of substances upon a parasite is bound up with all the questions of adsorption to the parasite-cell, with alteration in permeability, and eventually of storage in the parasite, and, above all, with alteration of the function of the parasite-cell through influence of its metabolism—in other words, with all the problems which we encounter in pharmacology.

It is perhaps possible that in the distant future investigators may succeed in killing off with one substance and at one stroke all the parasites which exist in a diseased host. Experience has shown that we may perhaps succeed in destroying only a fraction of the parasites whilst many others are merely damaged. And we know that by certain chemotherapeutic drugs, such as germanin, trypanosomes are not killed, but are only functionally altered, the body of the host completing the destruction of the parasite by its own defensive mechanism. The chemotherapeutic process of healing is therefore inextricably bound up with the life-process in the diseased organism of the host, either by the drug developing a more or less intensive effect upon the cells or organs of the host, or by the organism of the host completing the healing process started by the chemothera-

peutic drug (Schlossberger, 1937).

Reflection will show how undesirable it is for the investigator in chemotherapy to specialize too strictly, and how wide must be his general survey of the achievements of scientific investigation. In many cases, especially in the protozoa, the parasites have a definite developmental cycle. The chemotherapeutic drugs do not affect all the stages of such a development. Individual stages are often only susceptible in a specific way: for instance, quinine and atebrin have no effect on the gametocytes of *Plasmodium falciparum*, but it is just these forms which are damaged or killed by plasmoquine. Plasmoquine, however, has no effect upon the schizonts of the same form of malaria (cf. Roehl, Mühlens and Kikuth, quoted by Schulemann, 1935). We meet further fundamental differences in the behaviour of medicaments towards one and the same parasite, both in human beings and in various species of animals. Infection with trypanosomes in mice is totally different in its character and course from infection in cattle or human beings (Manteufel and Taute, 1930). Just as in pharmacology it is impossible, from an experiment on animals, to draw binding conclusions regarding the behaviour of a drug in practice, so it is impossible in chemotherapy. The very same methods of comparative investigation of drugs which are used in pharmacology must also be employed in chemotherapy, but in the latter case we require also to understand the biology of the parasite. The investigator in chemotherapy must therefore work in closest conjunction with the clinician if he is to attain results which are of practical utility.

I hope that I have shown how wrong it would be for the investigator in chemotherapy to specialize too narrowly. Only close co-operation with the varying branches of science, of biology, and of theoretical as well as practical medicine, can lead to success. That success can only be reached by means of hard and careful work is obvious. We must absolutely refuse to accept anything which savours of sensationalism, even though we are told that, after years of systematic work carried out by a large staff of co-operators involving the expenditure of much money, this or that substance has been proved to act specifically against this or that infection; even though we are told that many thousands of experiments on animals have been performed—and many such arguments are often used for purposes of advertisement—we must refuse to be deterred from con-

sidering the fundamentals required by chemotherapy.

By what procedure, then, can we attempt to discover new remedies for infectious diseases? (Cf. Fischl and Schlossberger, 1934; Schilling, 1938; Oesterlin, 1939; Findlay, 1939.) To try out new compounds on diseased human beings is in practice impossible. Our first attempts must be made in infectious diseases inoculated into laboratory animals, either by employing parasites which originate in diseased human beings and are also infectious for animals, or by working with micro-organisms only pathogenic for animals. So long as the circumstances allow, we use those laboratory animals which are not too costly and whose maintenance is not too expensive or difficult. Our aim is to create conditions in the laboratory which approximate as closely as possible to corresponding cases in practice. First, we determine the maximum dose tolerated by the host; then we make tests on the infected animal to ascertain whether the maximum tolerated dose influences the course of the infection, either in the direction of cure or otherwise. If we observe signs of an effect, we perform further experiments to determine the minimum effective dose, and we thus obtain the therapeutic index. Then we compare this index with those of other substances, either new ones or those already in use in practice. If a new substance proves to be superior to those already known, we test its pharmacological-toxicological effect as exactly as possible upon the usual experimental animals, again as far as possible in parallel with the drugs already used in practice, for it is only by such a comparison that we can determine whether the new preparation warrants further clinical examination. investigator in chemotherapy is no more able than the pharmacologist to calculate from the results of laboratory experiments the doses which should be used in the first experiment on man; but comparison with doses of known drugs makes it possible for us to determine initial doses for tentative testing on human beings or domestic animals.

All this sounds very simple, but it presupposes, if we are to proceed in this empirical way, either that we must set off into the unknown depending upon excellent intuition, or that we have at our disposal well-nigh limitless supplies of money for carrying out systematic year-long experiments. The past has shown that such empirical methods can lead to success. No one could foresee, for instance, that atoxyl had a therapeutic effect against infection by trypanosomes or spirochaetes, yet Wolfestan Thomas in 1905 proved that it was able to cure an infection of mice with trypanosomes, and in 1907 Uhlenhuth showed that it had also a certain effect on spirochaetes. And recently we have met with similar illustrative examples. Although chemotherapy is slowly being placed upon a scientific basis, and although Warrington Yorke has made most valuable contributions towards this end, yet Yorke himself has demonstrated that, contrary to all expectations, undecane diamidine exerts a definite effect on malaria infection of human beings (Glyn-Hughes, Lourie and Yorke, 1938).

Once such a starting-point has been found, further researches must be undertaken. The chemist varies the constitution of the fundamental substances and the new compounds are tested biologically in a purely systematic manner. If the chemist works in close contact with the biologist, the latter is able to give him valuable advice for further developments, provided that chemistry and physical chemistry are not absolutely unknown fields to him. How quickly progress is possible is shown by the development of salvarsan. Ehrlich cleared up the chemical constitution of atoxyl and so opened the way for Benda and Bertheim to develop further the chemistry of aromatic arseno-compounds. Comparative tests, both in vitro and in experiments on animals, led Ehrlich to observe that compounds of trivalent arsenic have a special therapeutic significance, and he was then able to direct the activity of chemists from pure systematics into paths which led relatively quickly to practical success. If we compare Ehrlich's work with the results in later decades of the intensive work in this field which led to the synthesis and testing of an enormous number of arsenical compounds, we can fully realize the importance of close and intelligent co-operation between the biologist and the chemist. I need only remind you, for instance, of the number of arsenic compounds which are described in the monograph of Raiziss and Gavron (1923): only tryparsamide and stovarsol (spirocid) have proved of practical importance. The significance of tryparsamide lies in the fact that it is effective in the second stage of sleeping sickness, in which germanin fails. Although spirocid has long been known, it only gained practical importance as an enterally administered prophylactic against lues infection, and as a remedy against amoebic dysentery, after it was discovered to be less neurotoxic than other aromatic arsenic acids. We find similar developments in other directions. For instance, Plimmer and Thompson in 1907 discovered the effect of tartar emetic—an antimony complex salt—on trypanosome infections, and so gave renewed impulse to the further development of therapy with antimony compounds, which were of importance even in antiquity and were rediscovered in the Middle Ages by Paracelsus. Again, it was the co-operation of clinicist, biologist and chemist-especially of Uhlenhuth and Schmidtwhich led to the creation and discovery of the therapeutically more valuable aromatic and other complex antimonial compounds (Schmidt and Peter, 1937).

Occasionally, chemical compounds are also constituents of natural drugs, as, for instance, quinine in Peruvian bark, whose specific action against malaria has long been known. The elucidation of its chemical constitution, and the attempt to synthesize new compounds effective against malaria, remained without success for many decades. Ehrlich's discovery that the dye-stuff methylene blue had an effect, although a very modest one, against *P. malariae* led to the construction of new thiazol compounds effective against malaria. The transference of the result of this work into the field of quinoline and acridine brought the first practical success in the shape of plasmoquine and atebrin (Schulemann, Schönhöfer and Wingler, 1932; Mauss and Mietzsch, 1933).

It would not be difficult to multiply these examples, but it is of more importance to explain the methods by which the work is continued to-day. In the past, drugs were discovered empirically. At first, improvements and developments could be made only very slowly. Real success was not attained until the exact sciences had been developed, and until investigations in anatomy, physiology and pathology had prepared the way for further developments.

I hope that I have made this clear in respect of pharmacology; we shall now see that it is also true in chemotherapy. The investigator endeavours to obtain insight into the mode of action of drugs against infectious diseases and to recognize connections, and is thus able to abandon dead systematics and to develop

his investigations intelligently.

The starting-point for these investigations is before our time, for Ehrlich, who with the discovery of salvarsan had achieved such a remarkable practical success in the drug treatment of an infectious disease, based his work upon determined theoretical conceptions; and for a long time afterwards medical-biological thinking was governed to a large extent by his side-chain theory. 'Receptores' in cells, in foodstuffs, poisons and drugs; 'haptophore' and 'toxophore' groups; 'nutriceptores' and the other terminologies referred to in this technical jargon, react upon each other according to this theory. They were generally regarded as chemical reactions. The reactivity of the side-chains was said to change according to the chemical constitution, and, with that, 'avidity' was also said to change.

These diagrammatic presentations, which were, moreover, schematically presented by drawings of suction-cups, tentacles, hooks, eyes and so on (Aschoff, 1902), certainly had their value at that time as hypotheses upon which to work. For the scientist of to-day, however, they are not enough. The possibility of illustrating biological processes in a manner apparently so clear and simple has unfortunately actually held up investigation, for very often processes problematic in themselves were considered to have been solved by the clarity of the scheme.

The chemotherapeutic work of those comparatively early times often started with the investigation of dye-stuffs (cf. Kiyono, Sugiyama and Amano, 1938),

whose specific distribution in the organism could be investigated more easily than that of colourless compounds or drugs. Moreover, therapeutical thought was dominated by the idea that a substance would act most potently in the places where it was stored in greatest amount. It was therefore obvious that much intensive work must be carried out to investigate the regularity of dye-stuff distribution in the organism. Further progress was made by Goldmann (1909-13), who, although dominated by the side-chain theory, so far departed from the traditional morphological-descriptive considerations as to stress strongly

the importance of function (cf. also Kuczynski, 1922).

In continuation of this work, Evans and Schulemann (1914, 1915, 1917), on the one hand, and von Möllendorff (1915, 1920), on the other hand, showed, independently of each other, and with different methods, in investigating a long series of acid azo-dye-stuffs, that absorption, distribution and excretion depend on the state of solubility of the dye-stuff, which, for its part, is conditioned by its molecular extent, its constitution and antecedents, and the 'milieu' of the solution. Substances which are heterogeneous in their constitution show similar biological behaviour if the physico-chemical properties of their solutions are similar to each other, as, for instance, carmine, azo-dye-stuffs, triphenylmethane dye-stuffs, iron-sugar, huminic acids, metals (gold, silver and palladium) in solution, and so on. Especially remarkable is the fact that all these dye-stuffs and colloidal particles migrate in the electric field to the anode, and consequently have a negative charge. But the properties of such solutions are greatly varied by the antecedents of the solution, the medium of solution and the 'milieu'; in other words, the degree of concentration, the temperature, the age, the presence of other electrolytes and colloids, and the addition of peptinizing agents greatly change the properties of the substances.

In opposition to the side-chain theory, which assumed reaction-mechanism of a purely chemical nature, the view was therefore adopted that the constitution of dye-stuffs conditions and their physico-chemical properties represent the

fundamental conditions for absorption, distribution and excretion.

But in 1916, at the beginning of these investigations, Schulemann (1917, pp. 120, 138) pointed out how decisive an influence is exerted upon the behaviour of the substances by the mixture of their solutions with the body-fluids, and he emphasized in particular that the recently discovered laws could only hold good for the distribution of acid dye-stuffs in the body of animals, 'but for the storing up of the dye-stuffs in the cells these conditions could not alone be decisive.'

The problems of distribution and storing must therefore be treated separately, and hence it is clear that a suitable distribution of the substances must occur if an elective storing is to take place in any part of the body or in any of the parasites distributed throughout the body. In later years this problem was investigated by Bennhold (Bennhold, Kylin and Rusznyak, 1938). While oxygen, linked with the haemoglobin of formed particles in the blood, is

transported from the lungs to the place of consumption, many dissolved substances entering the circulation or the body-fluids come into more or less close relation with formed and unformed particles of the plasma.

Very many dye-stuffs form aggregates with the groups of albuminous bodies contained in the plasma, that is, with the groups of the fibrinogen, of the globulins and albumins. But we are convinced that we have not to deal with three distinctly defined albuminous bodies, but in each case with a large number of very different, but very closely related, compounds. Sörensen, Raehlmann and others had already drawn attention to such formation of aggregates. Later, Bennhold discovered that the albuminous bodies of the serum on the one hand accelerated the diffusion of those dye-stuffs which diffuse either slowly or not at all, and on the other hand retarded the diffusion of rapidly migrating dye-stuffs. The acid dye-stuffs which were used formed aggregates with the albumins of the serum, and these aggregates in vitro in a gel of gelatine exhibit very similar degrees of diffusibility.

By these cataphoretic experiments Bennhold succeeded in analysing the processes precisely. He was able to demonstrate, for instance, that basic atebrin under normal conditions migrates to the cathode, but that when mixed with serum it forms aggregates with the albumins which migrate to the anode. If an excess of atebrin is contained in the solution mixed with the serum, then the atebrin-albumin aggregates migrate to the anode, but the excess of free atebrin migrates to the cathode. Similar facts were proved for many other drugs and dye-stuffs, and also for the aggregates formed with the globulins. Naturally, in the animal body these conditions are much more complicated. For instance, Hecht (1936) demonstrated that atebrin which aggregates in vitro with the albumins is, in the blood of living animals, almost completely adsorbed by the erythrocytes (Bennhold et al., 1938, pp. 267, 270), and he failed to find that under these conditions atebrin-albumin aggregates are formed. Again, we know that other compounds, such as germanin, form aggregates with the fibrinogen of the blood-plasma, and that at the same time the coagulability of the blood is inhibited; but in vitro germanin aggregates also with the albumins.

So we see the old experiences confirmed, that experiments on models may give some very interesting results, but that their meaning must be interpreted and evaluated with extreme caution.

Moreover, we are still unable to answer the question whether a separation of such aggregates, which are doubtless formed, takes place in the body, or whether the aggregates of drugs plus albuminous substances enter the cells together. In many cases it is probable that the drugs and the albuminous substances are taken in by the cells from the circulation. This opinion is supported by the inhibition or the alteration of the coagulability of the blood by germanin and related acid dye-stuffs, and especially by the experiments performed by Jancsó on surviving organs, to which I will refer later. Bennhold, however, considers that in most cases the substances are split off from the

albuminous aggregates and that only the drugs, and not the albuminous substances, permeate into the interior of the cell. Here, moreover, the fact may be mentioned that the formation and stability of such substance-albumin aggregates vary widely, and that such substances as urea, sugar and so on, do not aggregate with albumins at all. It is remarkable that if, as Bennhold suggests, formation of aggregates does occur, they seem to become more stable the larger the size of the molecules of the substances and the lower their dispersion-rate in solutions. In Bennhold's opinion, such observations emphasize further relationships which exist between the physico-chemical properties and the distribution of the dye-stuffs.

With regard to distribution through the living organism, these facts are, or can be, of fundamental significance. Pfaff and Herold (1937), for instance, succeeded in demonstrating in experiments with fluorescent substances that the aesculin, which is dissolved in the blood and does not aggregate with albumins, flows out or is pressed out at the places where the arterioles originate, or where the capillaries split off from the blood-vessels. On the other hand, uranin, which aggregates with albumin, leaves the blood-stream only by diffusion, probably after the separation of the uranin-albumin aggregates. When inflammation occurs, an increased permeability of the walls of the blood-vessel results, probably with an early escape of the unchanged substance-albumin aggregates.

We can see, therefore, of what practical importance is the condition of the tissues at the moment of distribution of substances.

Bennhold has demonstrated that the distribution of congo red is completely different from the normal if amyloid, which stores congo red intensely, is present, or if, as in luetic nephrosis, the conditions of excretion of congo red are altered because the possibility of formation of aggregates with albumins is diminished.

Vonkennel and Schmidt (1939) have made an important survey of the behaviour of the different prontosil preparations. Bennhold had already proved that the prontosil dye-stuffs aggregate with albumin. Under normal conditions these dye-stuffs of the prontosil series do not pass into the liquor cerebrospinalis, and the same applies in the case of ulirone, with its relatively big molecule. The same thing happens with the sodium salt of sulphanilic acid, the molecule of which is indeed relatively small but appears in solution as a 'Zwitterion.' Special properties in solution are possessed by such compounds as prontosil album, albucid (Dohrn and Diedrich, 1938) and related substances, the molecules of which do not differ materially in size from those of the sodium salt of sulphanilic acid. These compounds easily pass from the blood into the liquor cerebro-spinalis. If inflammation occurs, the overflow of substances of big molecular size is very much facilitated, even if the substance forms aggregates with albumins. In this case, big albuminous molecules, such as those of the diphtheria and dysentery toxins, may pass the blood-brain barrier, as Bieling

and Oelrichs (1937) claimed to have succeeded in demonstrating on mice infected with the spirochaetes of relapsing fever.

Now let us go back to the action of drugs on parasites. However obvious it may be that a drug can only exert direct action on the parasite if it comes into contact with the parasite itself, we must not consider it necessary that the therapeutic effect of a substance is dependent upon its being stored in the parasite in large quantities. The question of specific affinity and storage has occupied the attention of investigators for a long time, a fact which is more easily understood if we consider the results of vital staining with acid dye-stuffs in the healthy and in the diseased body.

Let us, for example, take the case of two mice, into the peritoneal cavity of the first of which a water-soluble lithium salt of carmine is injected and into that of the second a suspension of carmine. Although we have injected identical chemical compounds, their distribution through the body differs according to the condition of their solution. The water-soluble lithium salt of carmine penetrates the whole body and is chiefly stored up in the endothelium of the liver, spleen and bone-marrow, and in the tubuli contorti of the kidney (Kiyono, 1914). The suspension of carmine, however, appears as a precipitate on the visceral and parenteral part of the peritoneum, and does not penetrate throughout the body. The precipitate of carmine is gradually taken up locally by the histiocytes derived from the reticulo-endothelial system.

A distribution analogous to that seen with the water-soluble lithium salt of carmine is shown by the azo-dye-stuffs of the trypan blue series, and also by the isamin blue 6 B, derived from triphenylmethane; whilst a distribution similar to that exhibited by the insoluble carmine suspension is obtained by the colloidal solutions of metals. When, for example, a colloidal solution of palladium is injected into the peritoneal cavity of a mouse, a precipitate is formed on the surface of the peritoneum, similar to that which occurs after injection of the suspension of carmine. The suspension of the metal colloid is, however, much finer than that of carmine, so that we can inject this colloidal solution intravenously without the danger of the formation of an embolus. In this case, the colloidal particles are taken in only by those cells which are in direct contact with the blood-stream. They do not pass through the whole body by diffusion, as do the dye-stuffs of the trypan blue series, and consequently no storage takes place in the epithelium cells of the tubuli contorti of the kidney.

As an important result of these investigations, we must remember that Aschoff has grouped the cells of the body electively demonstrable in this manner under the collective term 'reticulo-endothelial system' (R.E.S.) (cf. Schulemann, 1931). Later, we shall deal with this 'organ,' which is widespread throughout the body, when we consider the influence of the natural defence forces of the host

on the course of infectious diseases.

It is not only dye-stuffs, however, which are taken up by the cells belonging to the R.E.S.: agents of infectious diseases, such as the bacilli of tuberculosis,

are phagocytosed by the cells. Evans, Bowman and Winternitz (1912, 1914) demonstrated this fact by their fine experimental investigations on the development of the bacilli of avian tuberculosis in rabbits. The bacilli which are injected into a mesenteric vein of the rabbit pass through the vena portae into the capillaries of the liver, where miliary thrombi are first formed. Afterwards the bacilli are phagocytosed by the Kupffer cells. They continue their development in the protoplasm of these cells, which eventually they destroy; at the same time they give rise to the new development of functionally equal derivatives of the endothelium, and these newly formed cells then intervene in the further development of the process: in this way the tubercle is developed. It is thus clear that the cells which phagocytose the tubercle bacilli are the same as those which store the acid vital dye-stuffs in largest amount.

It thus appeared that this simultaneous elective storage of substances in those body cells in which the agents of disease continue to develop after being phagocytosed, might provide a basis for the search for substances which would act therapeutically. But all experiments so far performed along these lines have failed, which is scarcely surprising in view of the nature of the storing process and the inert granules formed inside the cells, which very probably

should simply be regarded as inactive precipitates or aggregates.

But pharmacology has demonstrated that storage and action are two processes which do not necessarily go hand in hand. We know that alcohol, hypnotics and narcotics are not electively stored by those organs (for instance, the central nervous system) whose functional alterations are most clearly seen. At first there are certain differences in concentration in the various organs, but later these differences disappear. We see these differences clearly after the administration of alkaloids-atropine, for instance, which exhibits such a pronouncedly elective action on the parasympathetic nervous system. But we often find that such alkaloids are accumulated in the main in the liver, where they become inactivated. Furthermore, we may recall the observation of Straub (1907), that after some time a heart poisoned with muscarine regains its activity, even though the poison be contained in the food-solution in a still effective amount, but that the inhibition of the activity sets in again if the poisoned solution be replaced by food-solution without poison. From this Straub assumed that the poisonous action is caused by the ratio of concentration between heart-muscle and food-solution, or vice versa—a hypothesis which was later supported by other investigators and by other experiments (Rentz, 1929; Axmacher, 1938).

Of much greater significance than storage, for the influence of a substance upon the function of a cell, seem to be the processes occurring on the surface of the cells, in other words, the process of permeability, which we will now consider.

Though many acid dye-stuffs, after injection into a living organism, permeate through the cell-surface into the interior of the cells and are there

deposited in the form of granules, this process does not take place if a surviving organ is washed through with a solution of the same dye-stuffs or metal-colloids. Jancsó (1929, 1931) demonstrated that the livers of rats, first perfused with sodium chloride solution, decolorize a subsequently perfused colloidal solution of silver or gold. But the metals do not penetrate into the interior of the cells, but are merely adsorbed in the inside of the walls of the capillaries. The addition of human or animal serum to the colloidal solution before perfusion reduces the degree of adsorption, but absorption and storage in the interior of the cells of the R.E.S. now take place.

Even when the metals were at first merely adsorbed, subsequent perfusion with an albuminous solution was sufficient to transport the adsorbed metal into the interior of the cells. As a result of these experiments Jancsó came to the following conclusions:

In the living animal the plasma-albumins exert a fundamental influence on the storing process in the cells of the R.E.S. Not only do they inhibit the fixation of the colloids by adsorption to the inside of the walls of the capillaries, but they cause the Kupffer cells to function.

Although we are compelled to assume that the electivity of storage is not the only decisive factor for the effect of a substance, nevertheless these observations of Jancsó are of fundamental importance. They demonstrate clearly how the permeability of the cell-limits is dependent on the 'milieu.' The ability to penetrate into the interior of the cells is not only dependent on the properties of the substances and of the cells, but also on the colloids present in the blood or in the tissue-fluids. How great an importance is to be attributed to the problem of permeability has been shown quite independently from Jancsó's work. Investigations were carried out with different methods and ideas by Warrington Yorke and his collaborators in their classical work on the drug-resistance of strains of trypanosomes. Yorke and his collaborators (1931-38) proved that the lethal action of arsenical compounds on trypanosomes depends on the ability or otherwise of these compounds to permeate into the parasite. In this way they were able to prove that compounds of the arsenic and acridine series will be absorbed by strains of normal trypanosomes, which then succumb to their effect; but such compounds are not absorbed by strains which have been made resistant to them.

Independently of Yorke, Jancsó (1932) obtained similar results on normal and drug-resistant strains with the trypanocidal derivatives of acridine and of styryl quinoline.

This work of Yorke and of Jancsó stimulated new interest in the phenomenon of interference. As early as 1911, Morgenroth and Rosenthal had demonstrated that substances with little or no effect were able partially or completely to inhibit the therapeutic action of a substance. In the light of present-day knowledge, we may suppose that in such cases we have also to deal with variations of the cell permeability; and thus the phenomenon of interference is closely connected with the problem of drug-resistance.

In this connection the fact proved by Yorke seems to be of special interest, viz., that in many cases these variations of permeability, resulting in the production of drug-resistance, may be more easily produced *in vitro* than *in vivo*. A somewhat parallel observation was made by N. and H. von Jancsó (1935), who succeeded in producing drug-resistance more easily in the living animal after the reticulo-endothelium of the infected animal had been temporarily

inactivated by an injection of colloidal copper.

These investigations have been not only of great theoretical value, but of outstanding practical significance for the formation of drug-fast strains in the field-treatment of infectious diseases, especially of infections with trypanosomes. Up to the present, investigators have not succeeded in completely re-establishing lost permeability. Yorke has proved that the development of drug-resistance by trypanosomes is fundamentally the result of a process of mutation, although he could not exclude the possibility that, under certain conditions, the process of mutation may be aided by one of selection; and that the property of drug-resistance acquired by the trypanosome persists, even after the trypanosome has undergone its cyclical development in an intermediate host and after it has been passed through another species of vertebrate.

From the results of these investigations on the problems of permeability, we may, in my opinion, draw two special conclusions upon the mechanism of the action of drugs on parasites: firstly, it has been proved that for the production of drug-resistance the drug must penetrate into the interior of the body of the parasite* in order to act; secondly, we know from the work on interference that two substances totally different from one another may be influenced reciprocally in their ability to permeate. It is not necessary that these two substances should be therapeutically active: substances therapeutically

effective or ineffective may influence one another.

How, then, are we to understand the effect of the substances?

May I once more return to the problems of pharmacology. At the beginning of my lectures I drew attention to the fact that most of the variations of the function of cells are causally conditioned by an alteration of metabolism. These problems of metabolism are more or less specific for each species of cell, and for the function exercised by the individual cells or species of cells. Hence it is seen that drugs may act specifically and that the same drug may act in totally different ways on different kinds of cell. Moreover, the cells themselves do not behave only in a passive way. Under the influence of a substance the cells may change their permeability, and so be able to protect themselves against the influence of toxic drugs. That is true, for instance, in the case of arsenic-fast trypanosomes, as well as of arsenic-eating human beings with acquired tolerance

^{*}In this connection I may remind you of the fact, observed by Jancsó (1932), that in normal strains of trypanosomes the blepharoblast will be stained by, for example, acriflavine, but that this does not occur in drug-fast strains.

against arsenic oxide entirely owing to non-absorption of the drug (cf. Clark, 1937). But, once drugs or poisons have penetrated into the interior of the cells, they not only influence the processes of cell-metabolism, but themselves undergo the metabolic changes by which they may perhaps be activated or inactivated. For example, Loewi and Dale demonstrated the formation of active acetyl-choline from choline, its subsequent inactivation by saponification through esterase, and the influence of eserine in the process.

In chemotherapy we must also assume that drugs influence the cell-metabolism—which in our case means the metabolism of the parasite on which they are acting, and as a result of that action an alteration of the cell-function

occurs.

In pharmacology we endeavour to produce reversible alterations of the cellfunction, and to restore disturbed functions to normal in human beings or animals. In the drug therapy of infectious diseases we attempt to alter the parasite irreversibly, either by its total destruction, or by so influencing its development that the parasite either loses its ability to develop further or is damaged to such an extent that the defence forces of the host are able to complete its destruction.

In my opinion, it would be a mistake to attempt in any way to generalize on the mode of action of drugs. We shall be able to extend our knowledge of the mode of action only if we understand how to adapt our hypotheses and investigations to each single case.

May I consider some individual problems by means of examples:

Biot, Biot and Richard (1911) and Fleig (1911) have shown that, by the addition of glucose, the length of life of trypanosomes in culture is prolonged. Continuing this work, Schern (1925) proved that glucose is consumed in the metabolism of the trypanosomes. He also found that the amount of carbohydrate in the blood and liver of animals infected with trypanosomes is diminished immediately before the death of the animal, and this was later confirmed by other investigators. Savino's (1927) experiment of influencing the course of trypanosome infections in producing hypoglycaemia by the injection of insulin failed to furnish unequivocal results; but it proved that the presence of glucose is necessary for the life of the trypanosome, and that relatively large amounts of glucose are consumed in its metabolism.

From other experiments, Axmacher (1936), N. and H. von Jancsó (1934) and Issekutz (1933) came to the conclusion that the action of germanin is based

upon an inhibition of the carbohydrate-metabolism of trypanosomes.

Independently of one another, Jancsó (1935) and Schern (1911, 1925, 1928) investigated the course of infection with trypanosomes under the influence of substances which cause hypoglycaemia in the host. They found that synthalin (decamethylene diguanidine) is therapeutically effective, and they expressed the opinion that its effect is principally based on the fact that it causes hypoglycaemia, though they recognized the possibility that a direct action of the drug

might play a subsidiary rôle in the process. Later, Lourie and Yorke (1937) proved that the action of synthalin on the parasite is a direct one. Synthalin exerts a powerfully trypanocidal action *in vitro*, while insulin is practically ineffective *in vitro*. Continuing their investigations, King, Lourie and Yorke (1937, 1938) produced a long series of other guanidine-, amidine-, amineand isothiourea-derivatives and investigated their effect. Some of these substances proved to be highly trypanocidal in action, but there is apparently no connection between their effectiveness against trypanosomes and the production of hypoglycaemia. Though the experiments carried out with the object of influencing cell-metabolism in such a way as to produce a therapeutical effect have hitherto not succeeded, nevertheless there exists many reasons for regarding changes in cell-metabolism as causal for the action and effect of a drug.

We know that certain activators and inactivators influence the function of enzymes, without necessarily being part of the enzyme system itself. An especially well-known example of this is quinine, which not only acts against malaria infection, but is an antipyretic in general. As long ago as 1867, Binz, who worked as my predecessor in pharmacology at Bonn, inferred from his investigations on the action of quinine upon amoebae that very probably malaria was caused by protozoa—a supposition confirmed in 1880 by the discovery of the malaria parasite by Laveran. In 1873 Binz pointed out that quinine inhibits the formation of acid in the blood obtained by venesection. Later, Hoffmann (1877) proved that even small amounts of quinine are able to inhibit hippuric acid synthesis in the perfused kidney. In the following years much work was carried out which confirmed and analysed its restraining action upon enzymes (Laqueur, 1906). In the series of antipyretics there was for many years a certain special position attributed to quinine.

Pharmacologists believe that the lowering of temperature caused by quinine is effected not so much by the calming of the heat-centre as by the restraining of the oxidization processes—in other words, that quinine as an antipyretic acts chiefly through diminution of heat-production (Starkenstein, 1938, p. 533).

But again it would be wrong to generalize too far. The function of each enzyme is not necessarily decreased or increased by the same compounds. Their influence upon function is apparently quite distinct, and this specificity depends on the nature of the enzyme, on the nature of the substance, and finally on the 'milieu.'

For example, Rona and Petow demonstrated that the lipase of the pancreas is not influenced by atoxyl, but is inactivated by quinine. Esterase of the liver, however, is inhibited by atoxyl but uninfluenced by quinine; whilst strychnine, according to the work of Ammon and Fischgold, activates the saponification of the optically active left mandelicester by the esterase of the human liver. In this connection, the specific inhibitory effect of physostigmine against the esterase which saponifies the acetylcholine ester may be remembered (cf.

Ammon and Dirscherl, 1938). If we bear in mind the complicated process of glycolysis, which may be influenced more or less in its single phases, it will readily be realized how many single investigations are necessary and how wrong it would be to generalize.

Apparently Sir Rickard Christophers had such considerations in mind when he and his collaborator, Fulton, undertook their investigations dealing with the respiratory metabolism of malaria parasites and trypanosomes under normal

conditions and under the influence of drugs (1938).

But it is not only the carbohydrate-metabolism which is of importance for the life of the trypanosome. In 1911 Nauss and Yorke drew attention to the fact that oxygen disappears rapidly from blood containing trypanosomes, but that the production of carbon dioxide does not correspond to the great loss of oxygen. Fenyvessy and Reiner (1924) demonstrated acid production by trypanosomes, and showed that oxidative as well as glycolytic processes played their part in the break-down of sugar. And Reiner, Smythe and Pedlow (1936) were able to show the formation of pyruvic acid. None of these processes in trypanosomes is inhibited by the addition of potassium cyanide.

The behaviour of the malaria parasite is quite different, and the conditions are much more complicated, as the parasites are living in or on the erythrocytes. According to Sinton and Ghosh (1934), the parasites change the haemoglobin into haematin in the formation of the malaria pigment, and, moreover, there are different stages of development. With admirable clarity and skill, Christophers and Fulton (1938) overcame these difficulties in their classical work, and, in an irrefutable manner, were able to confirm the consumption of glucose, the formation of acid products and the great oxygen uptake by trypanosomes. Deprivation of glucose is followed at once by cessation of oxygen uptake. The reduction in pH, due to acid formation, appears to be the cause, even in the presence of glucose, of a gradual reduction in oxygen uptake. This falling off in O₂ uptake is corrected by the addition of alkali (buffer). All these processes are closely dependent upon the presence of living trypanosomes; after destruction of the trypanosomes the presence of an active enzyme could no longer be proved. Potassium cyanide is without any effect on the respiratory metabolism of trypanosomes.

On the other hand, the parasite-substance of *Plasmodium knowlesi* takes up oxygen even in the absence of glucose, and apparently quite independently of the glucose-content; it is clear that oxygen is obtained from the oxyhaemoglobin. This process goes on with great intensity, and with the very considerable consumption of oxygen there is an approximately equal output of carbon dioxide. The haemoglobin is altered, if not actually split up, the non-protein nitrogen is increased, there is no formation of acid comparable with that produced by trypanosomes, and no increase of ammonia can be detected.

Oxygen uptake is completely inhibited by potassium cyanide. It does not seem unlikely that the malaria parasite obtains oxygen mainly from the

haemoglobin of the host cell, and hardly at all from the serum. Fulton and Christophers have, however, not succeeded in elucidating the exact mode of action of drugs on trypanosomes or on malaria parasites.

The inhibitive effect of drugs on the oxygen uptake of parasites is dependent on the nature of the drug and of the parasite, and is proportional to the concentration of the drug. The degree of oxygen uptake depends upon the number of living parasites present, but how far abolition of respiration is equivalent to

death is not quite clear.

Christophers and Fulton are careful to point out that they have been unable to prove that the influence of drugs on the metabolism of the parasite-cell is due to an action on the enzyme (oxydases, dehydrases, carboxylases and so on), and they emphasize that further observations upon the enzyme-structure of the organisms dealt with are necessary before discussion of the effect observed can be of any value.

In the field of the arsenic compounds we meet with a similar development. Voegtlin, Dyer and Leonard discussed the question of the rôle played by the sulphydryl groups in the metabolism of parasites, and of how far the action of drugs on them is able to influence cell-respiration. Voegtlin and Smith showed that trivalent arsenic compounds probably do not act in the form of the arseno-compounds, but are oxidized in the body into aromatic arsenic oxides. These arsenic oxides are now believed to react with the sulphydryl groups of glutathione, whose function in metabolism as a redoxsystem would be inhibited in this way. Grassmann and his co-operators have shown that cysteine and glutathione activate cathepsine—the proteolytic ferment of the cell-plasma only in their reduced form. If these activators of cathepsine—as Bersin demonstrated especially for papain, which is closely related to cathepsine—are inhibited by oxidation or by combination with the arsenic oxides, the enzyme loses its activity. It is possible to reactivate the enzyme by reduction, by the addition, for example, of a hydrogen-transferring engine—of a dehydrase in presence of a hydrogen-donator. In the present case, therefore, we have to assume the coupling of the proteolytic process with processes of oxidoreduction taking place in the organism (cf. Ammon and Dirscherl, 1938; Oesterlin, 1939; Findlay, 1939).

In regard to the disturbance of the catalytic process of metabolism by drugs, the experiments of Jancsó (1936) seem to be of special interest. He was able to demonstrate that dye-stuffs of a distinct redox-potential may be able to interfere, quite independently of their constitution, with the therapeutic action of arsenical and antimonial compounds. Jancsó assumes that the applied dye-stuffs may be able for some time to replace the inactivated enzymes of metabolism.

Even though all these experiments have not yet succeeded in finding a final explanation of the actual mode of action of a drug, it seems certain that the final explanation and further developments lie in the direction indicated by the

experiments mentioned. Certainly much careful experimental work still remains to be done before we shall be able to draw any conclusions, and, as there are so many different processes concerned, any premature generalization on one observation would be extremely prejudicial to further development.

Moreover, it is not at all certain that every substance with parasiticidal properties acts on the metabolism by inhibition of the ferment-process in the interior of the cells. Investigations in the fields of interference and drug-resistance may perhaps suggest that the substances or drugs also inhibit the permeability of the cells for simple food-stuffs, in the same way as the substances may be able to hinder the uptake of drugs in the sense of interference. In such cases, the life of the cell would be disturbed by deficient nourishment or starva-

tion, and not by influencing the action of the enzymes of metabolism.

During my lectures I have frequently drawn attention to the fact that it is not only the properties of drugs, but the properties of the cell as well, which are of decisive importance for the action of the drugs. Accordingly, the susceptibility of various cells may be quite different under the influence of one substance. The more highly developed the parasite, the more numerous become the difficulties in the therapy of infectious diseases. Even with the protozoa we have often to deal with a whole cycle of development, and the single stages of development of one sort of parasite behave quite differently from one another under the influence of drugs. We can observe these conditions very clearly in the therapy of malaria infection. Until now it has been impossible to find a drug with a direct action against the sporozoites and against the first stages of development of these malaria parasites. The gametocytes of Plasmodium falciparum are practically unaffected by quinine and atebrin, but they are specifically influenced by plasmoquine. Missiroli (1938) has stated that certuna acts only on the gametocytes of P. falciparum and does not hinder the development of the gametocytes of P. vivax. A product able to destroy the endothelial forms of P. gallinaceum is as yet unknown.

With Entamoeba histolytica infections we find that drugs effective against the amoebae do not interfere with the development of the cysts. The same is also true for the different stages in the development of worms; the eggs, which are protected by a covering of chitin, are extremely resistant to the action of drugs. The same holds good for worms living in the parenchyma, in the gut or in other cavities of the body. The worm itself is a highly developed organism, and the pharmacologist has found that drugs act upon its different organic systems, e.g., by paralysing or exciting the musculature, by paralysing the nervous system, by destroying the integuments, and so on. Furthermore, the part of the body which the worm inhabits, and the manner in which it takes up its food, are of importance for the study of the action of drugs. Only if we take into account all these different factors may we succeed in our work.

However desirable it may seem theoretically, it is not necessary for practical purposes that the drug should kill the parasite in order to cure an infection.

In many cases even therapeutically effective drugs, such as germanin, do not exert a lethal action on the parasite. Germanin inhibits the division of trypanosomes and changes their properties in such a way that they can easily be phagocytosed by the cells of the R.E.S., and thus finally be destroyed. Similar facts are known of the action of emetine against Entamoeba histolytica. Under the influence of this alkaloid cell-division is disturbed. A further instance is given by anti-malarial drugs. Forms of degenerated schizonts are known to be produced by quinine, atebrin and plasmoquine a short time before the cells disappear from the blood. By small doses of plasmoquine, the vitality of the gametocytes of P. falciparum is so much reduced that they are unable to develop further in the stomach of the mosquito, though they may circulate apparently unchanged for many days in the blood of the infected organism. Regarding the action of certuna against the gametocytes of P. falciparum, Missiroli (1938) claims that the parasites may be able to continue their development in the stomach of the mosquito up to the stage of the ookinetes, and that then the parasite dies.

These reactions are not limited to the action of drugs against parasites. The alkaloid colchicine, for instance, is known to act specifically on cells by hindering their division shortly before its completion. The carcinogenic substances, on the other hand, are able to change the growing character of a cell, and to force it in another direction by causing a proliferation of cells in an atypical manner.

These examples demonstrate quite clearly that in the cure of an infectious disease many processes are working hand in hand. In the great majority of cases first the drug in some way or another damages the parasite and then the defence forces of the host enter the field and complete the victory.

In many cases these defence forces are able to cure an infectious disease without the co-operation of drugs. Although it is impossible here to consider the matter in detail, it is necessary to touch upon it in general terms. We must remember that in their mode of action and in their activity the defence forces of the host manifest considerable variations and that they may be of a specific or of a non-specific nature.

The specific defence forces are represented by immune bodies in the widest sense. The non-specific defence forces can be weakened or reinforced; consequently the age, race and general health of the host are highly important factors, which must never be overlooked in chemotherapeutic investigations.

During the last 20 years special attention has been devoted to the cells of the R.E.S., since they can be clearly revealed by vital staining with acid dyestuffs or with suitable solutions of colloids. Numerous experiments have been carried out to inhibit the action of the R.E.S., at least temporarily, in order to create simple conditions in investigating the direct action of drugs against parasites. By means of 'blockade' (surfeiting) of the cells of the R.E.S. for a

times with very large amounts of therapeutically ineffective compounds—sometimes combined with splenectomy—investigators have tried to inhibit the function of the cells of the R.E.S. By this means interesting results have been obtained; but on the whole such experiments have given only inconclusive results. It is difficult to state to what extent and for how long the cells of the R.E.S. are inactivated. Inactivation of the 'blocked' cells for, perhaps, only a short time will quickly be followed by an intense proliferation and activation of the cells in question. A period of inactivation of uncertain length is followed by a period of increased activity. Moreover, as the development of the parasites also requires time, it often remains a matter of uncertainty whether these two processes will synchronize.

A somewhat better prospect for success in inactivating the defence forces of the host was achieved by Jancsó's (1935) method of injecting a solution of colloidal copper, whereby the activity of the cells of the R.E.S. is eliminated for a longer period than is possible by 'blocking' them with inactive substances. But here, too, inactivation is followed by a phase of hyperactivity, so that by this method

also it has not been possible to obtain unequivocal results.

Finally, we have to remember that the defence forces of the host are not completely localized in the R.E.S.; this fact can be demonstrated by vital staining. Aschoff himself gave repeated warnings against the danger of overestimating the importance of the R.E.S.; we know, for instance, practically nothing about the function of the lymphoid system, which is distributed throughout the whole body and, at any rate in man, is much superior in amount to the R.E.S.

In this connection a paper of Menon (1939) may be mentioned, which deals with the alterations of the tissues occurring in monkeys infected with *Plasmodium knowlesi*. He demonstrates that not only does an activation of the R.E.S. take place, as evidenced by a differentiation of the histiocytes and an activation of phagocytosis, but that the parasites are localized in large numbers in the spleen by a vascular mechanism, and that a reaction of the lymphoid system also occurs in the spleen.

I hope that I have thus succeeded in demonstrating how many and how varied are the processes which must work together if we are to achieve our aim, viz., the cure of an infection. We can appreciate how many further investigations remain before we can attain to a fuller understanding of the mode of action of drugs. Even though what we have already learned may assist us in the continuation of our work, we still require intuition and, I might almost say, an artistic gift, in order to devise new methods and lines of research which will ultimately lead to success. For, in addition to the many obscure biological problems, our work is made more difficult by the fact that, until now, we have not been in a position to foresee how the variations of the chemical constitution of a compound may change its whole physico-chemical properties. We can only ascertain

this after synthetizing the compounds, and notwithstanding all our experience we often meet with the greatest surprises. Hence, we are forced to await the results of experimentation before we draw conclusions on the mode of action of a substance.

On the basis of experience and of the results of experiments hitherto obtained, we may speculate regarding further development. The closer and better the mutual understanding between chemist and biologist, the more successful their investigations will be, especially if the biologist possesses a wide knowledge in all the fields in which he has to work. It is his task not only to test the action of the substances manufactured by the chemist, but also to advise regarding the lines which further work should take. Besides all the routinework that must be carried out to establish the therapeutic value of a substance, it is necessary to probe deeply into the problems of the mode of action of drugs. Yet even then the task of an investigator in the drug therapy of infectious diseases is not finished. When a substance effective against infectious disease is discovered, all the methods of pharmacology and toxicology must be employed to ascertain whether the compound is suitable for practical use. In the course of these clinical investigations, many new problems and difficulties will arise which can only be overcome if the investigator in the laboratory is a good clinician and is able to act as a helpful adviser.

Finally, allow me to mention another technical point which is often of decisive importance for the value of a drug. That is the problem of the stability of a substance and of the possibility of its technical administration. We know how much the utility of tartar emetic is limited in practice by the fact that it cannot be given by the mouth or subcutaneously, but must be injected intravenously with great care in order to avoid the formation of local necrosis. A similar limitation is encountered with emetine. Quinine and the synthetically produced drugs effective against malaria would be of far less importance in practice if they could be administered only by injection. In many cases, however, it is both useful and necessary to give such compounds parenterally; it is well known how difficult it was to solve the problem in the case of quinine.

Mr. President, ladies and gentlemen, in these special University lectures in pharmacy I have thought it best to consider the general development of chemotherapy rather than to repeat special facts often previously reported. In following these general lines I have had an opportunity of acknowledging the fundamental work done in recent years by investigators in this country, and I shall indeed be glad if further co-operation in this friendly manner, which I appreciate very deeply, is continued in the future. In this way, we may contribute to further development in the drug therapy of infectious diseases, not only for the help of mankind, but for a further advance in mutual understanding. Please, Mr. President, accept my sincere thanks for the kind hospitality which I have enjoyed to-day, as so often before, at the Wellcome Research Institution.

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Prof. W. YORKE

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This number of 'The Annals of Tropical Medicine and Parasitology' completes vol. 33. Owing to war-time conditions it may be impossible in future to publish regular quarterly parts, but the editors hope to be able to issue numbers as regularly as possible.

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THE MALE AND THE EARLY STAGES OF ANOPHELES WELLCOME! THEOBALD

RY

D. J. LEWIS

(Agricultural Research Institute, Sudan Government)

(Received for publication June 27th, 1939)

The following descriptions of *Anopheles wellcomei* are based on the examination of 26 males, 21 pupae or skins, 19 larvae or skins, and several eggs. The eggs were laid by females collected near Kosti on the White Nile in January, 1939, and the different stages were bred from them.

The specimens are in the British Museum and the London School of Hygiene and Tropical Medicine.

ADULT MALE (fig. 1). Head. Vertex as in female. Proboscis dark, except on labella.

Palps. Outer surface of stem clothed mainly with flavescent scales; a few dark scales near junction of second and third segments and at apex of third. Club white with two dark spots. Numerous long pale hairs on inner surface of fourth segment.

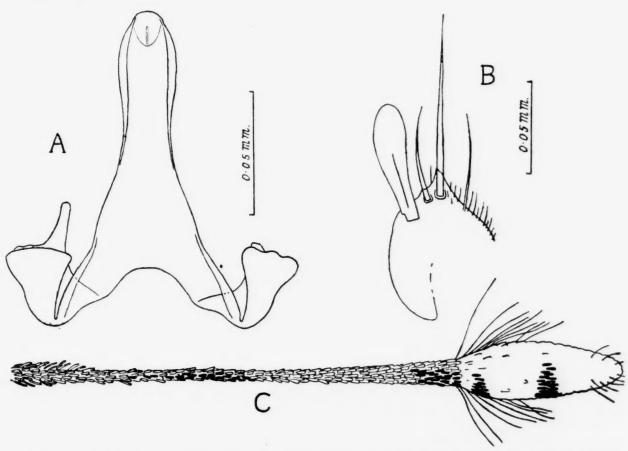


Fig. 1. Male of A. wellcomei. A.—Phallosome and dorso-lateral plates. B.—Harpago. C.—Palp.

Thorax, wings and legs. In general as in female.

Abdomen. Pale brown, flavescent scales on coxites.

Terminalia. Style with hair near apex about three times as long as width of style. Harpago with apical hair less than twice length of club. Outer accessory hair curved and about half the length of the apical. Inner accessory hair less curved and slightly longer than the outer. Phallosome without leaflets.

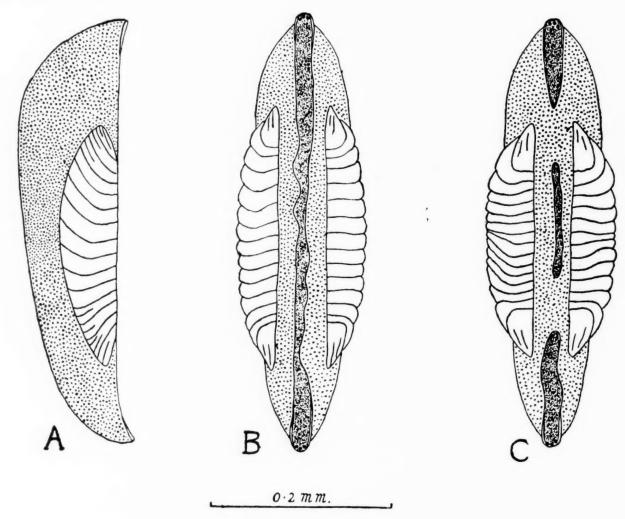


Fig. 2. Eggs of A. wellcomei. A.—Lateral view. B.—Dorsal view. C.—Example of variation.

Pupa. During life the pupa is pale green with conspicuous brown trumpets. It resembles that of A. distinctus var. ugandae Evans (Evans, 1938), except for the following points.

Spine A. IV, slightly longer than VII.

Bristle B. IV, 2 to 7 branches; III, 2 to 5 branches.

Bristle C. IV, 2 to 5 branches; III, 2 to 8 branches.

Cephalothorax. The row of smaller spines are as described by Evans, and are situated on the part of the pupal skin which covers the first pair of legs.

LARVA. This resembles the larva of A. theileri Edwards (Evans, 1938), except for the following differences.

Head. Fronto-clypeal pattern absent. Length of outer clypeal hair less than 1/3 to nearly 1/2 inner. Post-frontal hair simple or branched. Apical hair of antenna simple or bifid. Apex of antenna with internal spicular process of shaft longer than in A. theileri and about equal to that of A. distinctus var. ugandae.

Thorax. Inner shoulder hair broad and flattened, with about 22 to 28 branches. Middle shoulder hair with about 17 to 22 branches.

Abdomen. Palmate hairs with leaflets slightly shorter than in A. theileri. Teeth and plate of pecten without spicules.

The spicules which cover much of the larval integument are distributed as in A. theileri. They are especially long on the posterior lateral surface of the ninth abdominal segment between the saddle hair and the bases of the anal papillae. Here they are slightly curved and reach a length of 0.55 mm., slightly more than the diameter of the lip of the spiracle.

EGG (fig. 2). The length is about 3·2 times the breadth (including floats) and about 1·7 times the length of the floats, which have about 15 chambers. The floats are separated from the frill. The latter is narrow and usually continuous from one end to the other, enclosing a narrow area. It is generally twisted, and occasionally the frill of one side joins that of the other in several places, so that the enclosed area is divided into two or three parts. The upper surface is slightly more finely granulated then the lower, which shows no distinct polygonal markings.

ANOPHELES WELLCOMEI AND ALLIED SPECIES

The early stages of A. wellcomei have long remained unknown, probably owing to the habit of the larvae and pupae of creeping up objects projecting from the water and remaining in this position for long periods, so that in nature special methods are probably required for collecting them. The knowledge of their structure now clarifies the position of A. wellcomei with regard to the distinctus series. This consists of A. distinctus, A. wellcomei, A. schwetzi, A. walravensi and A. theileri, which were placed together on the strength of similarity in their wing-markings. The larvae and pupae of A. distinctus and A. theileri, and a variety of each, share several peculiar characters, and Evans (1938) wrote that 'It would be of great interest to know whether the larvae of wellcomei, schwetzi and walravensi also possess these characters.' The characters are described by Evans (1934, 1938), and A. wellcomei is found to possess all of them. Furthermore, the larvae and pupae of A. wellcomei resemble those of A. theileri and A. distinctus var. ugandae in their creeping habit. Another similarity between wellcomei and theileri is in their eggs, which possess the same general structure while differing in the size of the floats and in

minor points. The male of A. wellcomei, however, differs markedly from other known males of the distinctus group in possessing a phallosome without leaflets.

With regard to the status of A. distinctus var. ugandae, Evans (1938) wrote: 'If the early stages of wellcomei prove when discovered to be similar to those of distinctus, then this latter must be considered a variety of wellcomei, or it may be that ugandae should be regarded as a variety of wellcomei rather than of distinctus.' With reference to the size of the antennal spicular process, A. wellcomei evidently resembles ugandae more closely than distinctus, but it differs from both in several other larval and pupal characters; hence it appears that they should remain in their present systematic position.

I am much indebted to Professor P. A. Buxton for allowing me to carry out this work at the London School of Hygiene and Tropical Medicine.

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Lond.: Brit. Mus. (Nat. Hist.).

A CURIOUS ENTAMOEBIC STRAIN PARASITIC IN MAN

BY

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INTRODUCTION

Towards the end of 1937, Professor Schüffner called my attention to a patient whom he had treated for amoebic dysentery some years ago, and who, at that time, had produced entamoebic cysts which were very difficult to classify. Professor Schüffner was kind enough to give me much information about the patient, as well as the notes which he had made on the case and the stained slides which had been prepared early in 1937. From 1924 onwards, the patient had been under his observation, both clinically and parasitologically. In 1924 and 1925, there were attacks of amoebic dysentery; bloody mucus with large haematophagous amoebae was discharged, followed by typical cysts of Entamoeba histolytica, mono- and quadrinucleate, with cigar-shaped chromidia. On December 10th, 1925, the mean diameter of 44 cysts proved to be 13.25\mu. After a three-days' course of stovarsol the cysts disappeared early in 1926. In January and June, 1929, E. histolytica cysts were seen again; and at the end of the year the patient suffered from an attack of bacillary dysentery. Although E. histolytica cysts were again seen about this time, no amoebae were found in the bloody mucus discharged during the attack, and the cysts disappeared after a combined treatment of emetine and stovarsol. Frequent examinations in 1930 failed to reveal any cysts, and up to the time of writing no further intestinal complaints have been noted.

In 1932 and 1933 the stools were repeatedly examined, and at every examination showed typical cysts of *Entamoeba coli* with no abnormal characters. For the years 1934–36 no parasitological records were kept. On examining the stools again at the beginning of 1937, however, Professor Schüffner was immediately struck by the presence of abnormal cysts. He studied them for some time, noting their sizes and preparing stained slides, but abandoned his research when the cysts became very rare. In December, 1937, he left the case, with his notes and slides, in my care, at which time the cysts were again being produced abundantly. The information, etc., with which Professor Schüffner kindly furnished me, has been of very great value, as it has enabled me to conclude that the entamoebic infection producing these abnormal cysts already existed at the beginning of 1937, and that the character of the cysts has not in any way changed since then.

DESCRIPTION OF TROPHOZOITES AND CYSTS

TROPHOZOITES, which were fairly uniform in size $(18-22\mu)$, were seen only rarely, generally if the stools were semi-liquid or soft. The protoplasm was mainly endoplasma, sometimes with a narrow rim of ectoplasma. Usually the trophozoites were motionless; occasionally short blunt pseudopodia were extruded in an extremely sluggish manner. (Observations were made at room-temperature.) The endoplasma contained numerous rather small vacuoles. Bacteria were the only food-particles to be observed. The nucleus was usually visible in the living specimens; it was round, $4-5\mu$ in diameter, with chromatin granules close to the membrane. In stained specimens a small central karyosome was visible, and chromatic material close to the nuclear membrane (fig. 1, a). In short, apart from the greater mean size, the trophozoites closely resembled the 'minuta' forms of *E. histolytica*.

Cysts. *Number of nuclei*. In January and February, 1937, Professor Schüffner noted that the great majority of the cysts was mononucleate, and that there was a minority of binucleate cysts. The stained films made at that time showed the following details:

	No. of cysts with			
Date	l nucleus	2 nuclei	3 nuclei	
29.1.37	44	5	1	
1.2.37	48	1	1	
2.2.37	175	5	1	
4.2.37	47	3		
6.2,37	34	6		
Total	348	20	3	

In December, 1937, in 1938, and at the beginning of 1939, I repeatedly determined the number of nuclei with the help of a 1 per cent. iodine solution*; the results are shown in the following table.

Besides the cysts mentioned in the table, thousands were observed in stained films; these, too, were mostly mononucleate: binucleate cysts were always far fewer, and trinucleates were very rare. At the beginning of 1939, however, the proportion of binucleate cysts appeared to be increasing, and on two occasions

^{*}On January 24th, 1939, the determination was made in stained films.

TABLE

	D	ate		No. of cysts with l nucleus	No. of cysts with 2 nuclei	No. of cysts with 3 nuclei	Mean diameter in μ
1	937						
Dec.				35	õ	-	17.8
**	14th			113	10	1	17.6
11	15th			18	6	descriptions.	18-6
	20th			68	6	1	17:3
1.3	23rd			4.4	5	1	17.6
2.9	24th			45	4	1	17.1
2.2	28th			27	1		16.5
**	29th			39	8	3	18.2
2.8	30th			46	4	Million	17.9
**	31st	• • •	* * 4	11	3		18.1
1	938						
Jan.	3rd			30	- Macrosonia	-	17.3
11	4th			19	-	Anthonic M.	
,,	6th			72	3		
2.2	7th			55	1	Milheliagone	
31	8th			11	1	de man	
21	10th			15	1	Principle and	
11	12th			48	2	-	
19	14th			51	4		
,,	17th			38	Anthropping.	Badden man	
11	19th			3	- Contract	-	
2.2	21st			35	4	1	
22	27th			47	3	_	
,,	28th			49	1		16.5
Peb.	4th			12	2	Marries .	
1.5	5th			22	4	_	
1.2	8th			45	5	-	
1.2	9th			44	6	- Colomona	
12	10th			26	_	_	
2.5	15th			18	6	_	
3.5	16th		• • •	48	2		
,,	28th			41	7	1	18.0
lar.	12th		• • •	19	1	Addition	
11	15th			28	2	-	
,,	16th			41	4	MATERIAL STREET, STREE	
	22nd	* * *		45	4	1	
13	30th		• • •	25	3		
pr.	8th			10			
11	12th	• • •	* * *	27	4	_	
,,	13th			25	1		
Iay	5th		• • •	27	5	2	
,,	12th		• • •	15		1	
29	20th			48	1	1	

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TABLE (Continue 1)

Date		No. of cysts with I nucleus	No. of cysts with 2 nuclei	No. of cysts with 3 nuclei	Mean diameter in μ	
1938						
June 13th	• • •		50			
Oct. 24th	• • •		15	3	1	17.8
Nov. 15th	* * *	• • •	43	6	1	18.2
1939						
lan. 4th			6	4	-	
,, 24th			21	27	5	19.2
,, 27th			26	10		
Feb. 13th			11	12	2	
,, 22nd			98	10	3	
,, 23rd			65	6	1	
,, 24th			59	2	2	
Mar. 17th			20	4	*	
., 28th			54	5	4	
Apr. 6th			48	6	*	
,, 22nd		• • •	45	5	1	
Tot	tal		2,046	229	34	The state of the s

^{*}Also one cyst with 4 nuclei.

(on January 24th and February 13th) they even outnumbered the mononucleates; but this increase proved to be merely temporary.

Only two cysts with 4 nuclei were found in iodine solution, and two in stained films (fig. 1, n, o). Cysts with more than 4 nuclei were never found in direct microscopical examination of the stools; a 6-nucleate cyst was on one occasion observed in a culture tube.

The occurrence, although rare, of trinucleate cysts, together with the almost total absence of cysts with more than 3 nuclei, is a very particular feature. The relative size of the nuclei in 44 trinucleate cysts, seen in iodine solution as well as in stained films, was as follows:

- 2 large and 1 small nuclei in 34 cysts.
- 2 small and 1 large nuclei in 4 cysts.
- 3 nuclei of the same size in 5 cysts.
- 1 large, 1 middle-sized and 1 small nuclei in 1 cyst.

These cysts cannot be considered as potentially quadrinucleate with retarded division of one nucleus, for, if that were so, the majority would show one large and two small nuclei. In fig. 2 the nuclei of five trinucleate cysts, as found on January 24th, 1939, are outlined.

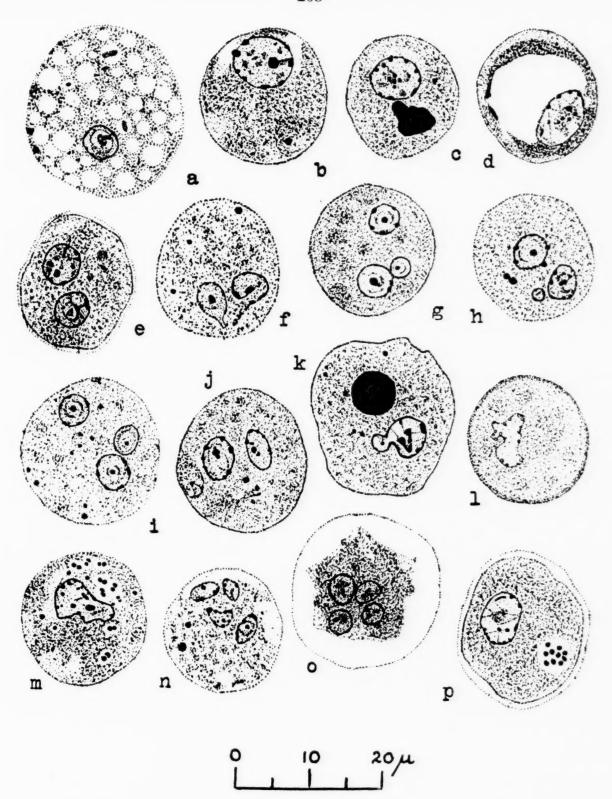


Fig. 1. a.—Trophozoite; b.—Mononucleate cyst; c.—Mononucleate cyst with chromatoid body; d.—Mononucleate cyst with large vacuole; e.—Binucleate cyst; f.—Binucleate cyst shortly after nuclear division; g.-i.—Trinucleate cysts; j.—?Trinucleate cyst; k.—Mononucleate cyst with chromatoid body and distorted nucleus; l.-m.—Mononucleate cysts with distorted nuclei; n.-o.—Quadrinucleate cysts; p.—Mononucleate cyst with ?parasites.

l shows a living cyst in eosine solution. a, c, d, m and p are stained with Hansen's haematoxylin; all other figures stained with Heidenhain's haematoxylin.

Size and structure of the nuclei. In the mononucleate cysts (fig. 1, b, c, d) the nucleus is usually very large $(7-8\mu)$, larger than in the trophozoite; in the binucleate cysts the nucleus is correspondingly smaller (fig. 1, e, f). Most of the chromatin is situated close to the nuclear membrane; a moderately large karyosome lies in the centre or somewhat excentrically. In Heidenhain-stained specimens the karyosome usually presents itself as a single dot (fig. 1, b); in Hansen-stained nuclei some four or five chromatic granules can be distinguished in it (fig. 1, c, d). Achromatic threads can often be observed in the nucleus of the mononucleate cysts. In the nuclei of the binucleate cysts there is often a slightly chromatic zone between the karyosome and the nuclear membrane (fig. 1, e). In the trinucleate cysts with one small and two large nuclei, the small nucleus may either be stained just as distinctly as the other two (fig. 1, e), e0 or be much paler (fig. 1, e1); in the latter case it is sometimes doubtful whether a third nucleus is really present (fig. 1, e1).

In mononucleate cysts the nucleus is often irregularly shaped (fig. 1, k, m). This is not an artefact due to fixation, for in fresh, living cysts the same phenomenon often occurred (fig. 1, l).

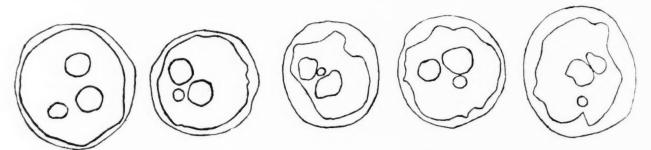


Fig. 2. Five trinucleate cysts as found on January 24th, 1939 (Heidenhain's stain).

The protoplasma of the cysts is homogeneous or somewhat granular. In the mononucleate cysts a vacuole is, as a rule, absent (fig. 1, b, c), though sometimes a rather large one is to be seen (fig. 1, d). The binucleate cysts very rarely possess a vacuole; on one occasion, however, in a 2-days-old culture, a cyst was observed with two nuclei and a very large vacuole, which occupied most of the cyst and gave it an appearance closely resembling that of a coli cyst. In most of the cysts glycogen is wholly absent. In some faecal specimens, however, cysts with a small amount of glycogen, as shown by the iodine reaction, are fairly numerous. A large vacuole well stocked with glycogen, as commonly seen in young E. histolytica cysts and especially so in young E. coli cysts, is extremely exceptional. Judging by the aspect of iodine films, the large vacuole sometimes seen in stained specimens contains little or no glycogen.

Chromatoid bodies were quite often met with in the cysts of some specimens; they were always either irregular in outline (fig. 1, c) or rounded (fig. 1, k)—never straight and parallel-bordered, like those in E. histolytica cysts.

Sometimes the protoplasma contains small darkly staining dots, either irregularly distributed (fig. 1, i), or arranged as diplococci (fig. 1, m), or in a group inside a vacuole (fig. 1, p)—in the latter case suggestive of *Sphaerita*, but without the characteristic concentric arrangement of those parasites.

Size of the cysts. Between January 28th and February 16th, 1937, Professor

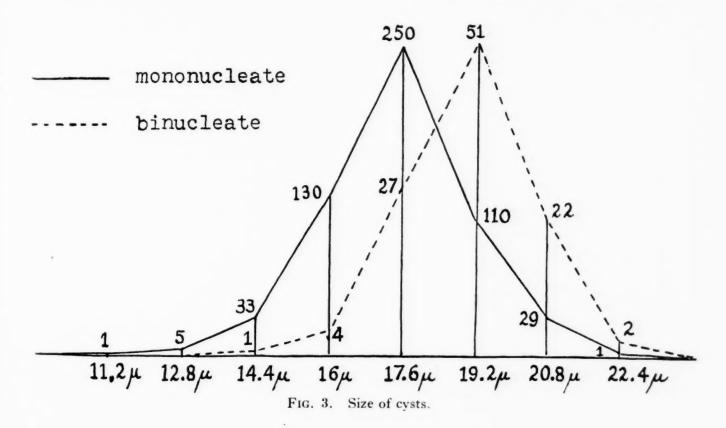
Schüffner measured 107 cysts and found : 12μ . . 1 cyst 15μ . . 10 cysts 13μ . . 2 cysts 16μ . . 20 cysts

10 cysts 18μ ... 26 cysts 20 cysts 19μ ... 18 cysts

 14μ .. 3 cysts 17μ .. 24 cysts 20μ .. 3 cysts

the mean size being $17 \cdot 1\mu$.

Between December 9th, 1937, and November 15th, 1938, I measured 559 mononucleate, 107 binucleate and 13 trinucleate cysts (in iodine solution). The measurements of the mononucleate and binucleate cysts are shown in fig. 3. The mean size of mononucleate cysts was 17.4μ , of binucleates 19μ ,



and of trinucleates 20μ , the mean size of all being $17 \cdot 7\mu$. The daily mean fluctuated between $16 \cdot 5\mu$ and $19 \cdot 2\mu$ (see table), this rather considerable difference being perhaps partly due to chance, as the number of cysts measured on one day was rather small. However, the table also suggests that a great percentage of the larger binucleate cysts raises the mean size—as might be expected. The almost perfect symmetry of both the curves in fig. 3 is a strong argument in favour of the homogeneity of the material throughout the time of observation.

ATTEMPTS TO CULTIVATE THE AMOEBAE

At the beginning of this research I believed that I was dealing with young immature cysts, and that sooner or later the appearance of mature multinucleate cysts would clear up the question of their nature. But this expectation was not fulfilled. And, since mature cysts are sometimes formed in amoebic cultures, cultivation was tried, especially when trophozoites were present, as former experience has shown that trophozoites are more promising material for starting amoebic cultures than cysts alone. Cultivation in various media was tried 28 times (once after the administration of a saline laxative), the solid part of the medium being coagulated horse serum or Difco's liver infusion agar, and the fluid part, horse serum, human serum or egg-white, diluted with Ringer's solution. In some tubes rice-starch, calcium carbonate, vitamin B₁, or combinations of these substances were added. In spite of this great variety of media, however, the result was invariably the same: the amoebae died in 2–6 days.

In some tubes, however, curious cysts were formed, $26\text{--}30\mu$ in diameter and having one large nucleus and a great vacuole. They were extremely rare, and, in all, I saw less than 10 specimens. Attempts to stain them failed, as I could not find them in stained films. In tubes inoculated with cysts only, excystation was never observed. On one occasion, in a 4-days-old culture I found, as the only survivor, a cyst that deserves to be recorded. It was $22\cdot4\mu$ in diameter; the protoplasm was clear, like water; it contained six large, round, equally sized nuclei, clearly visible in the living specimen. Had there been eight nuclei, or had two nuclei been larger than the others, I should not have hesitated to call it a *coli* cyst.

Hegner and his collaborators (1932) have recorded that development occurs in mononucleate cysts of E. histolytica after deposition, if they are kept at room-temperature. Although I am not altogether convinced that the procentual increase of bi- and quadrinucleate cysts observed by these authors cannot be explained by a greater mortality of the mononucleates, as well as by the latter becoming binucleate and quadrinucleate, the frequency of nuclear divisions noted in their material is in favour of their conclusion. I therefore attempted to obtain multinucleate specimens of my cysts in the following way. Faecal material containing mono- and binucleate cysts was well diluted with water and allowed to remain either at the temperature of the ice-box (9° C.), at room-temperature, or in the incubator (37°). In no case were cysts produced with more than two nuclei. Binucleate cysts became proportionately more numerous in the tubes kept in the incubator, but, as there was a heavy mortality, this may have been due to a longer survival of the binucleates, as well as to nuclear division in the mononucleates.

DISCUSSION

To summarize, we are dealing here with a strain of entamoebae producing large cysts, mostly mononucleate, less frequently binucleate, rarely trinucleate;

four 4-nucleate and one 6-nucleate specimens have been observed. The cysts rarely contain glycogen, and when it is present it is usually in a very moderate quantity. Sometimes irregularly shaped chromidia were seen. Observation of the strain has extended over two years, and so far the characters of the cysts have not altered. Diagnosis of an entamoebic strain should be possible after two years' observation, but in this case it was by no means easy. The first difficulty that arises is the question of whether or not the cysts, as observed, are mature. In cases of *E. histolytica* or *E. coli* infections loose or fluid stools may be passed containing, besides trophozoites, immature cysts only; normal well-shaped stools, if positive for cysts, hardly ever contain young and mature cysts or mature cysts only. In our case the stools were usually 'normal,' well-shaped, sometimes a bit loose, never liquid; hence, the assumption that all these cysts are immature *coli* or *histolytica* cysts is not in accordance with common experience.

Another difference between these cysts and those of *E. coli* and *E. histolytica* is the absence of glycogen from the great majority, and the fact that, when present, it is present in a small amount only.

Sometimes, in stained films, many cysts did not take the stain at all, a feature which I have never observed in *E. histolytica* cysts, though it occurs not infrequently in *E. coli* cysts. However, in the latter case it was the 8-nucleates that resisted staining, not the young (mono- and binucleate) ones.

It is true that the curious trinucleate cysts, with two large and one small nuclei, are rare, but they are a characteristic feature of the strain that has maintained itself throughout the time of observation. If trinucleates are observed in *E. histolytica* or *E. coli* infection, they usually show one large nucleus and two small ones.

Apparently infections similar to the present one are very rare, since no description has previously appeared after 25 years of intensive study of human intestinal amoebae by many competent observers. Its rareness suggests that it may be an animal parasite, accidentally and permanently established in man. Some entamoebic species parasitic in animals have been described, which produce mononucleate cysts only, or mononucleates and binucleates. Entamoeba chattoni Swellengrebel, 1914, from the monkey, the cysts show one, rarely two, nuclei. E. ovis Swellengrebel, 1914, found in sheep, produces mononucleate cysts only, as, according to Nieschulz (1923, 1924), does the porcine E. debliecki Nieschulz. (It is possible, however, that the quadrinucleate cysts found by Douwes (1921) in porcine faeces belong to this species.) Nieschulz (1922) described, without naming them, mononucleate entamoebic cysts from cow-dung. But in all these cases the cysts were smaller than 10μ (or 13μ in the case of Nieschulz's cysts from the cow). The cysts of E. suis Hartmann, 1913, another entamoeba parasitic in the pig, are, according to Nieschulz (1923), somewhat larger $(12-15\mu)$ and always mononucleate. It seems hardly possible to identify the present strain with any of these species. Apart from other differences, in most of them the cysts are much smaller. In E. suis the size of the cysts approaches that of our strain, but binucleate cysts were not observed in the former, whereas they are common in the latter.

To conclude, I should like to state that at present it is my personal opinion that we are here dealing with a new species. Further research on the case (which remains under observation) or on other similar cases must prove whether or not this is so.

ACKNOWLEDGEMENTS.—I am much indebted to Professor Schüffner for the discovery and history of the case; to Miss G. van der Meer for the drawings which accompany this paper; and to Professor Swellengrebel for help in its wording.

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ON A COLLECTION OF CESTODES FROM A PEACOCK (PAVO CRISTATUS L., 1758) FROM THE TERAI FOREST AREA, INDIA

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The material upon which the present paper is based was collected from the intestine of a male peacock (*Pavo cristatus* L., 1758) shot in the Terai Forest area, north of the River Sharda, Tatarganj District, Pilibhit, United Provinces, India, by the District Magistrate of Pilibhit, Mr. R. S. Symons, I.C.S., to whom the author is indebted for supplying the bird. The intestine was choked with parasites, only Cestoda being found. All measurements, except where otherwise stated, are given in mm.

FAMILY DAVAINEIDAE FUHRMANN, 1907 SUBFAMILY DAVAINEINAE BRAUN, 1900 Genus Cotugnia Diamare, 1893

Cotugnia longicirrosa sp. nov.

Length, 23-28; maximum breadth, 1.55. Scolex, 0.67-0.74 long and 1.28 broad. Rostellum, 0.23 diameter, smaller than sucker; rostellar hooks, 481-536, in double rows, 0.008-0.0085 and 0.012-0.018 long. On account of the thick musculature in the scolex, combined with a comparatively small rostellum, special care was taken to dissect out the latter; this greatly facilitated the recording of the number and size of the rostellar hooks. Suckers, 0.35-0.4 maximum diameter; acetabular hooks, 3-4 rows, 0.006 long. Segments usually broader than long, though a few terminal ones were comparatively longer. Genital pore slightly anterior to centre of proglottis margin, in some cases at anterior corner. Genital cloaca present. Musculature consisting of 7-8 rows of longitudinal muscles with minute and scattered fibres; transverse muscle fibres not observed. Cirrus sac, $0.36-0.51\times0.035-0.04$, extending well past the ventral longitudinal excretory vessel, slightly obliquely displaced towards the anterior margin of the proglottis. Vas deferens greatly coiled. Testes, 89-100, maximum diameter 0.07, in a band posterior to the level of the genital pore, extending across the proglottis from one ventral longitudinal excretory vessel to the other, and often directly dorsal to these vessels, or even external to them. Testes entirely absent anterior to ovary. Each ovary a much-lobed crescent, the concavity directed posteriorly. Ovaries close to, or at a little distance from, the ventral longitudinal excretory vessels, the intervening area usually being occupied by a few testes; in one case the ovary of one side overlapped the other in the centre of the proglottis, thereby forming a single mass. Eggs not completely developed.

In one specimen, cirrus sac, vagina and ovary of one side showed considerable variation from the usual position, being placed at a much lower level in the

lower one-third of the proglottis margin.

The present form is easily distinguished from species of the same genus which have the testes arranged in two distinct groups. Of the species with testes in a single continuous band, C. cuneata Meggitt, 1924, C. fila Meggitt, 1931, C. januaria Johri, 1934, C. joyeuxi Baer, 1924, C. meleagridis Joyeux, Baer and Martin, 1936, C. parva Baer, 1925, and C. seni Meggitt, 1926, are separated by their smaller number of testes (18-75); C. crassa Fuhrmann, 1909, by the

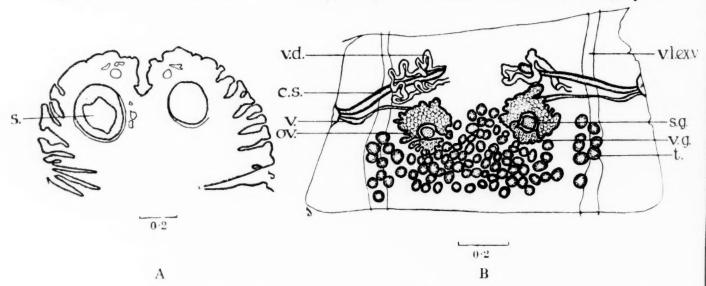


Fig. 1. Cotugnia longicirrosa sp. nov. A.-Longitudinal section of scolex. B.-Mature proglottis.

ac.h., acetabular hooks.t.,testis.c.s., cirrus sac.v.,vagina.ov., ovary.v.d.,vas deferens.s., sucker.v.g.,vitelline gland.s.g., shell gland.v.l.ex.v.,ventral longitudinal excretory vessel.

larger number of testes (150–200) and smaller cirrus sac (0·16); C. rimandoi Tubangui and Masiluñgan, 1937, and C. ilocana Tubangui and Masiluñgan, 1937, by the smaller number of rostellar hooks (300–320) and smaller cirrus sac (0·17–0·28); C. brotogerys Meggitt, 1915, C. fastigata Meggitt, 1920, and C. meggitti Yamaguti, 1935, by the relative and absolute size of the cirrus sac (0·29–0·32) and the uniform size of the rostellar hooks; C. digonopora (Pasquale, 1890) by the relative extent of the cirrus sac (not reaching the ventral longitudinal excretory vessel—about half the distance between the lateral margin and the excretory vessel). C. margereta Beddard, 1916, is comparable to the present form in that the rostellum is smaller than the sucker, but it is easily distinguished by the extension of the testes in the anterior portion of the segment, specially

anterior to the female genital organs, and by the relative extent of the cirrus sac. C. fuhrmanni Baczynska, 1914, described from the same host, with approximately the same-sized cirrus sac, is characterized by the smaller number and the uniform size of the rostellar hooks, together with the relative extent of the cirrus sac. The following table clearly indicates that the two forms are not identical. It is therefore necessary to list the present species as new.

	Cotugnia fuhrmanni Baczynska, 1914	C. fuhrmanni Baczynska, 1914, per Joyeux and Baer, 1936	C. longicirrosa sp. nov.
Length of worm	60-80	60-80	23-28
Breadth of worm	2.5	$2 \cdot 5$	1.24-1.55
Scolex diameter	0.56	0.56	1.28
Scolex length	0.4	?	0.67-0.74
Sucker diameter	0.182	0.18	0.35-0.40
Acetabular hooks	Very small	Very small	3-4 rows, 0.006 long
Rostellum breadth	0.086	?	0.23
Rostellar hooks (no.)	170	170	481–536
Rostellar hooks; size in μ	15	15	8-8-5 and 12-18
Genital pore	Mid third of lateral margin	;	Anterior to middle of lateral margin, often in anterior corner
Muscles	Longitudinal muscles, 2 layers Transverse muscles, 3 layers	?	Longitudinal muscles, 7-8 layers Transverse muscles, not observed
Testes (no.)	60-70	60-70	89-100
Cirrus sac	Located in lower half of segment	?	Located in anterior half of segment
Cirrus sac; size in μ	470×39	470×40	360-510 × 35-40
Cirrus sac (extent)	Slightly median to longitu- dinal excretory vessels	?	Well past longitudinal ex- cretory vessels
Ovary	1. In anterior portion of seg- ment, approaching anterior border 2. Lateral margins almost touching excretory vessel		In mid portion of seg- ment, far away from anterior border Lateral margins not touching excretory vessel

Cotugnia taiwanensis Yamaguti, 1935

Maximum length, 176; greatest breadth, 3.5. Scolex, 0.59 maximum diameter. Rostellum, 0.3 diameter; approximately 465 rostellar hooks, 0.01–0.012 and 0.014 long. Genital pore in anterior half of proglottis margin. Ventral longitudinal excretory vessels fairly wide and very well developed. Cirrus sac, $0.17-0.24 \times 0.032-0.045$, nearly approaching but not touching the ventral longitudinal excretory vessels. Testes, 86–89, in two groups. Egg capsules, 0.056-0.062; eggs, 0.042-0.05; and onchospheres, 0.018-0.029 in their maximum diameters.

The above description differs from that of Yamaguti in various details, but not sufficiently to justify the creation of a new species.

Raillietina Fuhrmann, 1920

Raillietina (Paroniella) symonsii sp. nov.

Length, 116; greatest breadth, 0·6. Scolex, 0·117–0·12 diameter. Rostellum, 0·063 diameter; rostellar hooks, approximately 110–120, 0·006 and 0·008–0·009 long. Suckers, 0·039–0·042 diameter; acetabular hooks very well developed, 0·007–0·012 long, in 8–9 rows. Genital pore unilateral, slightly

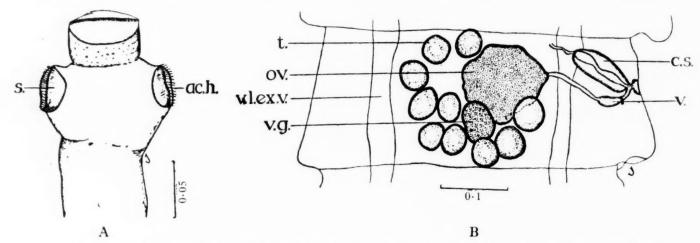


Fig. 2. Raillietina (Paroniella) symonsii sp. nov. A.—Scolex. B.—Mature proglottis. (Lettering as in fig. 1.)

anterior to the middle of the proglottis margin. Genital cloaca poorly developed. Cirrus sac, $0.1\text{--}0.12 \times 0.04\text{--}0.046$, extending to the ventral longitudinal excretory vessel. Testes, 10--11, 0.04--0.056 maximum diameter, surrounding the ovary laterally, posteriorly, and with a few anteriorly. Ovary slightly poral. Egg capsules numerous, $0.05\text{--}0.062 \times 0.03\text{--}0.051$, not extending lateral to the ventral longitudinal excretory vessels.

The specific number of testes of the present form distinguishes it from all other forms of this subgenus, except the following:

It is easily separated from R. biroi (Kotlán, 1920) by the longer cirrus sac

(0.16) and the smaller number of egg capsules (34-36); from R. facile Meggitt, 1926, by the small number of rostellar hooks (85) and the relative and absolute size of the cirrus sac (0.18); from R. tragopani (Southwell, 1922) by the smaller number of rostellar hooks (46), together with their uniform size and a very long cirrus sac (0.25); from R. numida (Fuhrmann, 1912) by the large number of rostellar hooks (160-180), the greater number of the rows of the acetabular hooks (15) and the peculiar disposition of the cirrus sac (oblique and nearing the aporal margin of the proglottis). R. fulvia Meggitt, 1933, approaches the present form in the size of the cirrus sac, but is easily distinguished by its extension (not to the ventral longitudinal excretory vessel), by the disposition of the testes only lateral to the ovary, by the extension of the egg capsules lateral to the excretory vessels, and by their large size $(0.092-0.19 \times 0.074-0.086)$. The details of R. blanchardi (Parona, 1898) from rodents (the description of which is incomplete) and of R. conopophilae (Johnston, 1912) from Passeriformes are not accessible to the author in Rangoon. The author has great pleasure in naming this form after Mr. R. S. Symons.

Raillietina (Raillietina) volzi (Fuhrmann, 1905)

Length, 50; breadth, 1·16. Scolex, 0·365 maximum diameter. Rostellar hooks, 210, 0·016–0·018 long. Suckers, 0·144–0·159 diameter; no acetabular hooks were observed. Cirrus sac, 0·11–0·14 long; vas deferens greatly coiled. Testes, 26–30. Ovary very well developed, its anterior region extending up to the anterior border of the segment. Egg capsules, 64–70, each containing 9–12 eggs.

The absence of acetabular hooks, the smaller size of the cirrus sac, and the smaller number of egg capsules are the main differences from the previous descriptions of Fuhrmann (1905) and Johri (1934); they are, however, not of sufficient importance to justify the exclusion of the present specimen from this species.

Raillietina sp.

Length, 85; breadth, 0.95. Scolex, 0.285 diameter. Rostellum very small, 0.036 diameter; rostellar hooks, 127–139, 0.008 and 0.01 long. Suckers, 0.095–0.134 diameter; no acetabular hooks observed. Genital pore unilateral at anterior half of the proglottis margin. Cirrus sac, 0.093–0.106 \times 0.026–0.03, extending to the ventral longitudinal excretory vessel. Testes, 29–32, 0.044 maximum diameter, surrounding the ovary on all sides except its poral border. Ovary slightly poral, approaching the poral longitudinal excretory vessel. Gravid segments entirely absent.

It is impossible to assign this worm to a definite species until further material is available.

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EXPERIMENTS ON THE UTILIZATION OF SUGARS BY MALARIAL PARASITES

(PLASMODIUM KNOWLESI)

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Bass (1911) announced that he had been able to keep alive and cultivate the three common forms of malarial plasmodia. Sinton (1912) repeated this work without success, using P. vivax and P. falciparum, but before the details of Bass's technique were available. Thomson and McLellan (1912) were able to cultivate one generation of P. falciparum, as some details of the method had meanwhile been announced in a paper by Bass (1912), in which the author states that, in the majority of cases, glucose must be added to the medium in order to secure growth of the parasites. In a further paper by Bass and Johns (1912), in which full details of technique are given, the statement that glucose is a necessity for cultivation is qualified. The authors record that, of a number of carbohydrates tested, including cane sugar, lactose, galactose, dextrin, mannitol and maltose, only the last appeared to be as useful as dextrose for culture purposes. In this paper the addition of 0.1 c.cm. of 50 per cent. sterile glucose is recommended to be added to 10 c.cm. of infected blood so that parasites may remain viable. Such procedure is always carried out in this laboratory when blood for infection purposes is sent out, and the blood with P. knowlesi parasites so treated has proved infective in America 12 days later.

In the present communication is recorded an investigation, by respiratory methods, of the power of *P. knowlesi* to oxidize various sugars. It was hoped by this means to supply an answer to the question as to which sugars could best be employed in culture experiments—a field which has been little explored. Two types of materials have been used for this purpose. The first type, in which the parasites were isolated in bulk (Christophers and Fulton, 1938), was washed in Ringer solution to free it from serum and accompanying glucose, so that apart from reserve material, possibly glycogen, the only substrate available was that which had been added to the medium in which the oxygen uptake was measured. A large number of sugars of varied composition have been studied in this way. The other material employed was that in which the parasites had been freed from red cell material by saponin (Christophers and Fulton, 1939).

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During the course of the experiments, observations have been carried out at the same time on the blood-sugar values and liver-glycogen content of rhesus monkeys suffering from acute infections of *P. knowlesi*, which reach a phenomenal intensity. The phosphorus metabolism of the parasites has also been studied during incubation at 37° C. in presence and absence of glucose, in an attempt to find out if phosphorylation occurs during the utilization of that sugar by the parasite.

MATERIAL AND METHODS IN RESPIRATION STUDIES

The preparation of fresh parasite material in bulk, as well as of saponized material, has been fully described in the papers already quoted (Christophers and Fulton, 1938, 1939). In the first method of investigation employed, 2 c.cm. of the material, typical differential counts of which are given in a later section, were suspended in 8 c.cm. of Ringer solution and centrifuged, the operation being carried out three times. A 0.25 suspension of the washed parasite substance was then made in M/15 phosphate buffer pH 7.4, and 1 volume of the latter was mixed with 2 volumes of Ringer containing 0.3 per cent. of the sugar under examination. In the case of the standard tubes the sugar was omitted. Oxygen uptake measurements were made in Barcroft manometers at 37° C., using 3 c.cm. of the above mixtures in each flask on the right-hand side. The controls on the left-hand side were similar, except that parasites were absent. The oxygen uptake was noted at the end of each 15-minute interval over a period of one hour, and the mean value for three manometers was used in each case.

The sugars tested were of varied constitution and may be grouped as follows:

Aldopentoses Xylose, arabinose.

Aldohexoses Galactose, glucose, mannose.

Ketohexose Laevulose.
Trihydric alcohol Glycerol.
Tetrahydric alcohol Erythritol.
Pentahydric alcohol Adonite.

Hexahydric alcohols Dulcitol, mannitol, sorbitol.

Hexahydroxy-cyclohexane or Inositol.

Methyl pentose Rhamnose.

Disaccharides Maltose, sucrose, lactose.
Trisaccharides Melezitose, raffinose.
Polysaccharides Glycogen, dextrin, inulin.

Phosphoric esters Hexose, di- and mono-phosphate (Robison ester).

Nucleotide Adenylic acid.

Amino-sugar Glucosamine.

Glucoside Salicin.

Only glucose, laevulose, maltose, mannose and glycerol caused an increase in oxygen uptake when added to the medium in which the washed parasites were respiring. The glucose used was a Kahlbaum specimen of the highest purity. The laevulose and maltose were Pfanstiehl preparations; the former from inulin had a specific rotation of -92° , and the latter of 131° . The glycerol was a B.D.H. 'analar' preparation, and the mannose, from the same firm, was also a pure specimen of melting-point $135-136^{\circ}$ C. and specific rotation $14\cdot3^{\circ}$.

In Table I below are recorded the actual oxygen uptakes in presence and in absence of sugars in each case, as the observations were made with different fresh preparations of parasite material. Similar oxygen uptake measurements were made with the red cells of a normal monkey treated in the same way as the parasitized cells. There was, however, no increased uptake by the red cells in presence of sugars which had caused increased uptake in the case of parasitized cells. That glucose actually does disappear in the medium was verified. Approximately 800 million parasites used 2.91 mgm. of glucose in $1\frac{1}{2}$ hours, while in the case of normal red cells the disappearance of glucose was found to be negligible.

On examining the formulae of the sugars which are oxidized by the parasites it is seen that all possess the grouping H - C - OH

This fact has already been noted by Kudicke and Evers (1924) for trypanosomes. These authors studied the power of certain sugars to support the life of *T. brucei* by observing movement in the culture medium over a period. The results obtained in the present investigation with *P. knowlesi* have shown that the same sugars are utilized. It would seem reasonable, however, to suppose that any sugars which the parasites are able to oxidize would be attacked at the aldehyde group. Apparently, however, the presence of a free aldehyde group in any particular sugar does not ensure oxidation, or otherwise we should expect to obtain an increased oxygen uptake with a number of those tested. This is supported by the fact that, whereas maltose is oxidized by *P. knowlesi*, lactose with a similar free aldehyde group in the glucose part of the molecule is not. The necessity for the presence of certain groups or a particular spatial configuration in the sugar molecule before it can be oxidized by the malarial parasite is, for the moment, only a matter of conjecture. It is a noteworthy fact that glycerol caused the greatest increase in O₂ uptake amongst the active substances.

In the second method of investigation the parasite host-cell was removed, as it is liable to complicate the interpretation of the results obtained. The free parasites so obtained, or some of them, were viable, as they gave rise to an infection on inoculation which ran the normal fatal course in rhesus monkeys. Only these sugars were investigated, with the saponized material, which had caused an increase in oxygen uptake with washed parasites. In each case there was again an increase, but of lesser degree. The parasites freed from their

host-cell had undergone severe treatment, as, after incubation for half an hour at 37° with saponin in saline, they were washed in Ringer solution and centrifuged three times to remove haemoglobin completely. When treated with saponin directly in the Barcroft apparatus, so as to remove all traces of red cell material, the diminution in oxygen uptake is very small when compared with that of untreated material in the control flasks. It is well known that the white cells of the blood are able to utilize glucose and to give rise to an oxygen uptake. In the present experiments, however, leucocytes were present only in the proportion of 1 leucocyte to 500 infected cells or less, and in the saponized material were

Table I

O2 uptake in mm.3 by 3 c.cm. of 0.25 suspension of washed and saponized parasite substance in presence and absence of various sugars, measured at 15-minute intervals over 1 hour

Time	Washed material												
in minutes	Glu	cose	Glyc	cerol	Laev	ulose	Malt	cose	Manr	nose			
15	7.8	56-2	3.3	49.0	8.2	53.8	6.7	12.1	5.8	26.2			
30	15.4	102.5	7 - 1	98.2	15.5	101.2	14.5	23.8	10.4	48-1			
4.5	19.3	138-2	9.0	146.7	$27 \cdot 6$	130.5	18.4	32.4	13.5	68.9			
60	24.1	162.4	11.7	194.7	33.7	151-2	22.9	39.0	17.0	79.7			
					Saponize	d materi	al						
15	4 · 4	18.7	6.6	31.5	6.4	32.8	8.2	10.0	4.5	5.:			
30	7.2	34.2	10.5	60.6	8.9	58.3	12.1	20.1	6.6	8.6			
45	9.5	42.8	14.7	78.8	12.6	74.8	15.0	25.6	8.9	12.6			
60	11.0	52.9	16.0	94.2	15.4	$86 \cdot 2$	18.3	30.7	10.6	15.8			

The first column under each sugar shows the uptake by the parasites in absence of sugar, and the second that when sugar had been added to the medium.

obviously so damaged or absent that they could not contribute in any way to the results obtained. Estimation of the glucose present before and after $1\frac{1}{2}$ hours in the Barcroft apparatus showed that approximately 800 million parasites free from red cell used $2\cdot 2$ mgm. of glucose. This value is comparable with that obtained above with washed cells. The values obtained for oxygen uptake by saponized cells are recorded with those for washed cells in Table I.

It will be noted that in the case of the washed material, which presumably has little oxidizable material accompanying the parasite, the increase in oxygen uptake on adding the sugar is considerable, but that it is not so marked in the case of saponized material which has undergone long manipulation. In the

case of maltose and mannose the uptake is actually small. Since glucose has proved most useful in culture experiments and maltose is a possible substitute, as indicated by experience, it would appear probable that those other sugars which cause an increase in oxygen uptake could serve a similar purpose.

BLOOD-SUGAR AND LIVER-GLYCOGEN IN MALARIA

It has previously been shown by Christophers and Fulton (1939) that P. knowlesi can cause a rapid disappearance of added glucose in vitro. Bloodsugar values were recorded for about 20 monkeys with heavy P. knowlesi infections, along with those for a number of normal animals. It was then concluded that such values were lower in the case of infected animals and became progressively more so as the infection advanced. This view has been substantiated in the present investigations. In human malaria the reports on blood-sugar levels are conflicting, and the values recorded show wide variations according to whether blood samples were taken before, during or after a paroxysm. results of different authors are not in agreement. Rhesus monkeys do not experience paroxysms, and the infection usually proceeds by stages to a fatal termination. In the present investigations most of the animals were chronic cases harbouring latent infections, and relapses were brought about by removal of the spleen. By this means heavier infections were obtained than in animals suffering from a primary attack. The blood-sugar values have been found correspondingly lower in the splenectomized animals. It is improbable that absence of the spleen could have been the sole cause. The sugar values estimated by the method of Hagedorn and Jensen (1923) are recorded, along with other relevant data, in Table II below, for 17 splenectomized and 5 non-splenectomized animals, all with heavy infections. Values for two control animals are also given.

Sinton and Hughes (1924), Williams (1927) and Green (1929) have shown that the liver in many cases of malaria is unable to store glycogen in the normal manner. The various contributory causes have been reviewed by Sinton and Kehar (1931). The sugar metabolized by the parasites may act as a continual drain on the liver-glycogen store. On the other hand, damage to liver cells by malarial infection, and consequent interference with function, is a common finding at autopsies. Lack of appetite and therefore of intake in these infected animals, as the fatal termination approaches, is doubtless also a contributory cause, although some animals eat normally till they are killed for the purpose of obtaining parasite substance. Liver-glycogen estimations were therefore made on the above series of animals at the time of killing, to find if the gravity of the infection bore any relationship to the amount of glycogen in the organ. The data obtained are summarized in Table II.

The method of estimating glycogen was essentially that of Pflüger. About 10 gm. of liver were chopped up and added to 15 c.cm. of 60 per cent. caustic potash immediately after the death of the animal. The flask and contents

TABLE II

Giving blood-sugar and liver-glycogen values of splenectomized and non-splenectomized animals suffering from heavy *P. knowlesi* infections, along with those for two control animals. The ratio of parasitized to non-parasitized cells and to whole blood was obtained from the volume relations after centrifuging the blood as drawn for $\frac{3}{4}$ hour, at 2,000 r.p.m.

Splenectomized animals

Mean value	Percentage of parasitized cells to blood volume	Percentage of parasitized to non- parasitized cells	Liver- glycogen per cent.	Blood-sugar; mgm. per 100 c.cm.	Monkey no.
	16	58	0.56	88	124
	20	75	0.29	44	142
	7	21	0.90	74	146
	12	46	0.12	51	147
	13	42	0.36	21	148
	4	14	0.32	89	149
Sugar 53	13	56	0.14	42	151
and an	25	74	1.14	24	152
Glycogen	8	28	0.15	46	155
-1,008011	8	20	0.75	104	156
0.66	9	29	0.56	67	158
	13	38	2.67	100	160
	20	400	0.09	16	163
	11	45	0.20	32	164
	10	30	2.62	56	166
	13	40	0.20	25	168
	23	113	0.10	27	170

Non-splenectomized animals

12	28	0.21	56	18	
167	97	0.56	33	8	Sugar 9
171	88	0.48	18	5	Glycoger
173	109	1.25	20	7	Sugar 98 Glycoger 0.62
175	155	0.61	20	3	

Control animals

Sugar 90		 7.9	98	3
Glycogen	nionna	 7.5	81	159
7.7				

were then heated in boiling water for 6 hours. At the end of that period the contents of the flask were neutralized with hydrochloric acid and made up to a known volume. The precipitated protein was then filtered off, and an aliquot portion of the clear filtrate, generally 10 c.cm., pipetted into a 50 c.cm. centrifuge tube. Two volumes of absolute alcohol were added to the latter, and the glycogen thereby precipitated, along with some sodium chloride. On standing 18 hours precipitation was complete. After centrifuging, the supernatant fluid was removed and the precipitate washed in absolute alcohol and then in ether, followed by centrifuging each time. The solid was then dissolved in 25 c.cm. of 2·2 per cent. hydrochloric acid in the same tube and the glycogen hydrolysed to glucose by heating on the water-bath for 4 hours. The mixture was neutralized on cooling, and the glucose estimated as above; and hence the amount of glycogen was calculated.

Glycogen was also estimated in dried parasitized and non-parasitized cells from the same monkey, after separation by means of the centrifuge from whole blood. The amount of glycogen found for the former was 0.24 per cent. and for the latter 0.20 per cent. These values are not very different, but they indicate a slightly increased glycogen-content in those cells containing parasites. It is possible that it may serve as a reserve food material. Previously, much lower glycogen values were reported, but they had been calculated on the weight of wet material. The present values for the parasitized and non-parasitized cells

are strictly comparable.

From the results recorded in the table it is seen that the blood-sugar values of the splenectomized animals are lower than in the non-splenectomized, most of the latter having less severe infections. This is in general true also for liver-glycogen, but nos. 160 and 166 show abnormally high values and raise the mean value considerably. In both types of animal the lowest value for liver-glycogen corresponds to the lowest value for blood-sugar, but at the same time no constant relationship exists between them. Also, there is no definite relationship between these values and the ratio of infected to non-infected cells, or between the ratio of infected cells to total volume of blood. The liver-glycogen values in the case of control animals are very much higher than those found for infected animals. It is not yet clear whether the low glycogen-content of the livers in infected animals is due to loss of storage capacity, to large demands made on blood-sugar by the parasites, to reduced intake of carbohydrate or to some other cause.

PHOSPHORUS CHANGES DURING METABOLISM

In order to find out whether phosphorylation of glucose takes place during metabolism of this sugar by malaria parasites, the following experiments were carried out. The parasite substance obtained from a number of monkeys, a representative percentage differential count of which is given below, was, in the case of 146 and 147, washed in a large excess of 2 per cent. citrate and

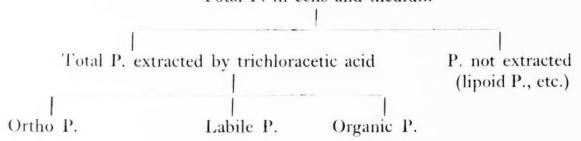
then centrifuged to free it from serum. The same operation was carried out three times in the case of 152. Saponized material in which the parasite is entirely freed from red cell (Christophers and Fulton, 1939) was obtained from 152 and 148, haemoglobin being removed by repeated washing in Ringer solution. Normal red cells were obtained from animal 3.

PERCENTAGE DIFFERENTIAL COUNTS

			No. 146	No. 147	No. 152
Full-grown schi	zonts		 43.6	26.0	34.0
3 ,,	,,		 $52 \cdot 2$	62.0	51.0
$\frac{1}{2}$,,	* *		 1.0	6.8	5.8
Rings and small	form	S	 1.0	0.4	3.6
Leucocytes			 0.0	0.6	0.2
Uninfected red	cells		 $2 \cdot 2$	$4 \cdot 2$	5.4

A 25 per cent. suspension of the parasite material was then made in 2 per cent. citrate, and to 1 volume of the suspension was added, in one case, 2 volumes of a mixture from 96 c.cm. of citrate and 4 c.cm. of M/15 phosphate buffer pH 7·4, containing 4·136 mgm. P. per c.cm., and to another a similar solution, which contained in addition 0·3 per cent. of glucose. To 15 c.cm. of each mixture was added 5 c.cm. of water and 6 c.cm. of 20 per cent. trichloracetic acid, and the precipitated proteins were then filtered off. The same process was carried out after incubation of the mixtures for 5 hours at 37° C. The various phosphorus fractions were then estimated in the clear filtrate according to the following scheme:

Total P. in cells and medium



Total P. was estimated by the method of Stewart and Hendry (1935), ortho P. by that of Briggs (1922) and labile P. by hydrolysing an aliquot portion of the acid filtrate in N/HCl, in a sealed tube at 100° for 10, 20 and 30 minutes —complete hydrolysis was, however, obtained at the end of 10 minutes. Total ortho P. in the hydrolysate was estimated as above, and the amount of labile P. is given by the difference in ortho P. before and after hydrolysis. The organic P. is calculated as the difference between total P. and the sum of the ortho and labile P. These results are given in the table below. Measurement of oxygen uptake made on the mixtures with and without glucose showed a marked increase in uptake when this sugar was present.

It is known that normal red cells contain phosphorus of which about 25 per cent. is hydrolysable, and the phosphorus changes recorded for these cells are in the same direction as for infected cells. In the saponized substance all red cell material is, however, absent, and the parasites, or some of them, so obtained appear to be alive, as they have proved infective on inoculation. In the washed infected cells there is a considerable increase in total acid soluble phosphorus after incubation, which was not found to be the case for normal cells. Ortho phosphate also shows large variations. For both types of material there is a decrease in organic phosphorus on incubation, while the values for

RESULTS OF P. ESTIMATIONS GIVEN AS Y P. PER C.CM. OF ACID FILTRATE

				Without addition of glucose										
Monkey no.		146		147		152		152		148		:}		
Total P.		• • •	51.4	66-8	52.9	63.2	49.2	59.8	51.3	61.2	41.6	57.0	44-1	45.0
Ortho P.			35.4	58.0	36.0	57.5	35.6	47.2	46.8	50.4	36.5	40.0	34.2	41.7
Labile P.			8.2	5.6	4.4	3.8	1.2	2.7	1.8	2.8	0.6	1.5	2.3	0.8
Organic P.	•••		7.8	3.2	12.5	2.9	12.4	9.9	2.7	8.0	4.5	15.5	7.6	2.5
						,	With a	ıdditio	n of g	lucose				
Total P.	* * *		52.8	64.8	53.7	66.0	49.3	60.0	51.3	61.5	42.1	58.8	45.0	47.7
Ortho P.			34.8	55.0	35.7	57.6	35.8	48.4	46.2	50.0	36.6	39.8	34.5	31.0
Labile P.	• • •	•••	7.0	8.0	4.7	4.1	0.8	1.1	3.8	2.4	0.8	3.0	2.8	4.4
Organic P.			11.0	1.8	13.3	4.3	12.7	9.0	1.3	9.1	3.7	16.0	7.7	12:

The first column under each monkey number gives the P. values before, and the second those after, incubation. In the case of 152, the first set of values are for thrice-washed material and the second set for saponized substance.

labile phosphorus remain fairly constant. The increase in total acid-soluble P. and part of the increase in the ortho P. must result from hydrolysis, during incubation, of one or more substances not originally extracted by the acid, and part of the increase in ortho P. is due to hydrolysis of acid-soluble organic P. Thus hydrolysis presumably occurs during incubation to give ortho P.

In the saponized material, on the other hand, there is a marked increase in organic P. on incubation, which may be accounted for by phosphorylation of one or more substances. The small increase in ortho P. on incubation may

possibly arise from the metabolism of phospholipins. One could then expect to find differences in the inorganic P. content of the sera of normal and infected monkeys. This point was investigated, using for P. estimation the method of Fiske and Subbarow (1925), but no differences were found in the two types of sera, and, in fact, both types showed considerable variations amongst themselves (3.96–6.70 mgm. P./100 c.cm.).

The evidence for phosphorylation of glucose during parasite metabolism appears to be lacking, as the various P. values recorded in presence and absence of this sugar are practically the same. Tests were, of course, made to see whether, by the method of Briggs, phosphorus in a phosphorylated sugar was directly estimable. It was found that the normal colour failed to develop till hydrolysis by heat in the acid medium had occurred. Indications that phosphorylated sugars are not metabolized by malaria parasites have already been given.

SUMMARY AND CONCLUSIONS

A large number of sugars have been studied by respiratory methods with the object of determining, amongst other things, whether they could serve as nutrients in culture experiments with *P. knowlesi*. Both washed and saponized parasite material was used. It was found that:

1. Glucose, laevulose, maltose, mannose and glycerol were oxidized by the parasites, as indicated by an increased oxygen uptake.

2. These substances have a common chemical grouping in their molecule which does not include a ketonic or aldehydic group.

3. Glycerol caused the greatest increase in oxygen uptake with both types of material.

Since glucose and maltose have proved of value in culture experiments with human malaria parasites, there is strong presumptive evidence from the results obtained that the substances mentioned above should act in a similar capacity with *P. knowlesi*.

The blood-sugar level in monkeys with heavy *P. knowlesi* infections is in most cases much lower than in normal animals, and the liver-glycogen store in these animals is also much depleted. The contributory causes have already been discussed.

As a result of estimations made of the different phosphorus fractions during metabolism of glucose by *P. knowlesi*, it is concluded that there is no clear evidence of phosphorylation of this sugar by the parasites.

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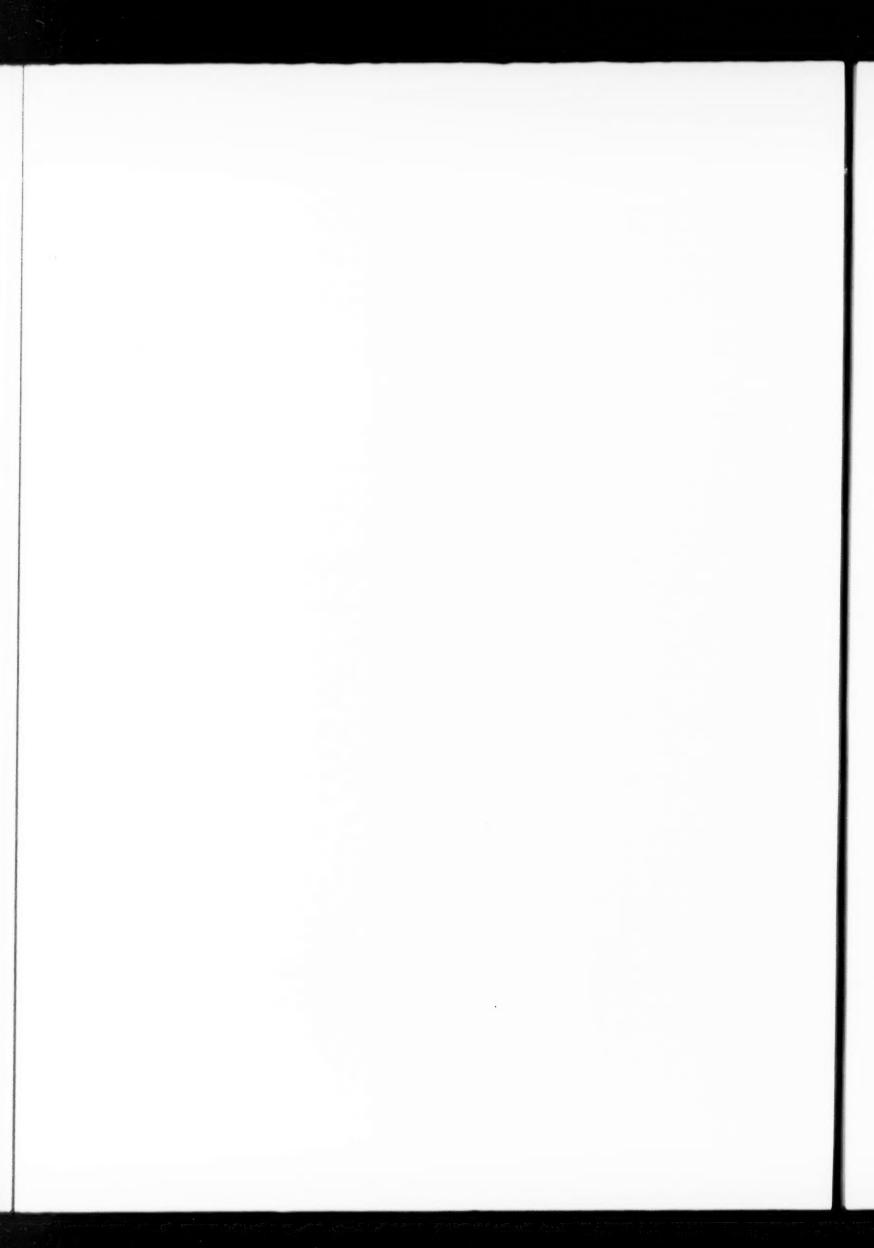
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THE STRUCTURE OF THE CAPITULUM IN ORNITHODOROS: A CONTRIBUTION TO THE STUDY OF THE FEEDING MECHANISM IN TICKS

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INTRODUCTION

In this paper, the descriptions of the capitulum or 'false head' of Ornithodoros are based chiefly upon a study of living specimens and preserved material of O. moubata (Murray); but, having in view certain publications by Sen (1934, 1935, 1937), an examination has also been made of the capitulum of O. tholozani (Lab. and Mégn.) from preserved material. Sen's work includes observations upon a number of Ixodid ticks, but his description of the capitulum is based on material of O. papillipes Birula (O. crossi Brumpt)*.

The capitulum in ticks has been studied by numerous authors. Several genera of the Ixodid ticks are dealt with by Nuttall, Cooper and Smedley (1905), Allen (1905), Bonnet (1907), Nordenskiöld (1908, 1909), Nuttall, Cooper and Robinson (1908), Samson (1909), Patton and Cragg (1913) and Ruser (1933).

The capitulum of Argasid ticks has been most fully described by Christophers (1906) in his work on O. savignyi (Audouin), by Robinson and Davidson (1913a, b) in their studies upon Argas persicus (Oken), by True (1932) who examined O. coriaceus Koch, and, latterly, by Sen in a series of communications of which one (1935b) constitutes an account of the capitulum of O. tholozani (L. and M.) (syn. papillipes B.). Although the present writer is mainly concerned with the capitulum in Argasid ticks, it has been found that the general relationships of the component parts of the capitulum of O. moubata

^{*}The material of O. tholozani (L. and M.) used by the present writer was supplied from a laboratory-strain bred by Professor S. Adler of the Department of Parasitology, Hebrew University, Jerusalem. Professor Adler notes in his correspondence relating to the supply of ticks that, according to Brumpt, the species is O. tholozani (L. and M.) (syn. papillipes Birula). Specimens received from Professor Adler have been sent to Mr. R. G. Whittick of the British Museum, who, in the present circumstances, has been able to give only a provisional opinion upon this point in synonymy. He does, however, consider papillipes as synonymous with tholozani, although the reservation is made that this opinion is based only on general appearance, the form of the camerostome, the arrangement of the discs, and the shape of the tarsal humps. The information at our disposal seems, therefore, to justify the use throughout this paper of the name O. tholozani (L. and M.) in preference to O. papillipes B.

and O. tholozani are similar to those of the Ixodid and the Argasid ticks discussed in the papers cited. There are, however, certain modifications of the capitulum described herein which appear to be absent in other ticks, or which have not been fully reported upon by previous authors. It may here be noted that the account of the capitulum of O. tholozani (Sen, 1935b) suggests that Sen, while observing one of the modifications detailed in this paper, offers an explanation of its structure and significance differing fundamentally from the interpretation given by the present writer.

In the description which follows the nomenclature used by Robinson

and Davidson (1913a, b, 1914) has been adopted.

Technique. The present account is based upon whole mounts of opaque and cleared capituli, mounts of parts of the capitulum, and serial sections at $10-15\mu$ in the transverse plane and in the longitudinal vertical and horizontal planes of the capitulum. With the exception of the serial sections, all preparations have been mounted in glycerine jelly, a hollow-ground slide or a cell ring being utilized in the case of complete capituli. In studying the details of the exoskeleton it was necessary to dissolve the soft parts by steeping the capituli for 24 hours in 10 per cent. NaOH. The cleared capituli were then washed in water and transferred to 10 per cent. glacial acetic acid for 24 hours. Capituli being prepared for whole mounts were, after clearing, placed in 70 per cent. alcohol for 10-14 days and then stained in Mayer's alcoholic cochineal for at least 10 days. It was found that the destaining which occurred during the subsequent passage of the specimens to water gave adequate differentiation. Soaking of the specimens in glycerine and in lactophenol preceded the final mounting in glycerine jelly. In the preparation of cleared stained parts of the capitulum, e.g., the hypostome, the same technique was repeated, and the required parts were dissected out when the stained capitulum was in glycerine. Capituli for serial sections were cleared in 10 per cent. NaOH and stained in Mayer's alcoholic cochineal as described above, or by a modification of Bethe's stain (Gatenby and Painter, 1937). The modification consisted of using saturated solutions of the reagents which were allowed to act for 15-20 minutes or longer. By this method, greater speed was obtained in staining, and the resultant deep blue-green colour gave sharp outlines to the sections. In some instances, sections were stained on the slide in eosin in absolute alcohol. Various reagents, including xylol, xylol and chloroform, and chloroform and carbon disulphide, were employed for clearing prior to embedding, but most satisfactory results followed the adoption of Peterfi's methyl benzoate technique (Carleton and Leach, 1938). Specimens were embedded and cut in paraffins melting at 52° C. and 58° C.

In addition to making observations upon material macerated with NaOH, it was considered desirable to check such observations by comparing them with sections through capituli which had not been subject to treatment resulting in a loss of the soft parts. For this purpose capituli were dissected, under saline,

from ticks (O. moubata) killed by momentary immersion in boiling saline. dissected capituli were fixed in Zenker's fluid in the usual way, and transferred to 70 per cent. alcohol, in which they were soaked for about 10 days. they were stained in borax carmine for a similar period. Destaining followed in 70 per cent. alcohol, and it was found that 2-3 days in this alcohol, together with the passage of the specimens to absolute alcohol, gave satisfactory differentiation and a convenient density of staining of the soft parts in the final sections. In addition, a number of ticks were killed, immediately after a meal on a rabbit, by immersion in boiling saline. The capitulum was dissected out in each case, fixed in hot 70 per cent. alcohol and passed rapidly to paraffin. Sections were cut at a thickness of 10μ and were stained on the slide in a mixture of carbol xylol and eosin, which served only to differentiate muscle masses from chitinous structures. In earlier attempts to obtain sections including the musculature, capituli were dissected out and fixed in hot Kleinenberg's picrosulphuric acid mixture, and were subsequently sectioned before staining on the slide with Ehrlich's haematoxylin and eosin. Valuable sections were obtained by this method, but experience has shown that, in staining sections of tick capituli on the slide, there is a considerable risk of important parts of the sections becoming washed off. For this reason, it proved desirable to avoid staining techniques which necessitated numerous transfers of sections on slides through different reagents. Embedding methods were various, but Peterfi's technique was again found to be the most satisfactory.

The greater part of this research has been done on female ticks, but a few males and late nymphs have also been used. No significant differences have been detected between the structure of the capitulum in the females, males and late nymphs.

THE CAPITULUM OF O. MOUBATA

Although, as stated above, the capitulum of *O. moubata* is essentially similar to that of other Argasid ticks, it is necessary to give an account of the capitulum and its component parts in order to understand certain details of structure involved.

The capitulum projects from a depression, known as the camerostome, which is situated antero-ventrally on the body of the tick (fig. 3). The general form of the capitulum may be likened to that of a filter funnel, the broad end representing the base of the capitulum or the basis capituli, and the stock indicating the position of the mouth-parts.

The mouth-parts consist of, ventrally, a median hypostome flanked on each side by a four-segmented palp, and, dorsally, a pair of long subcylindrical shafts, known as the chelicerae, which pass posteriorly into what Christophers (1906) aptly describes as 'a conical prolongation' of the basis capituli (figs. 1–3). The tubular space between the dorsal and ventral components is discussed more fully below.

The haemocoele of the body is continuous with the cavity of the basis capituli and extends to the distal extremity of each of the five mouth-parts. Posteriorly, the exoskeleton of the basis capituli flexes forward to become continuous with the wall of the camerostome.

The *hypostome* projects forward as a ventro-medial process of the basis capituli. It attains its maximum width at its point of attachment to the basis capituli, and terminates distally in a broadly rounded tip. In section, the dimensions of the hypostome decrease from a somewhat rectangular outline at the base (fig. 8) to a relatively thin plate of chitin at the tip (fig. 4). The ventral surface is convex and bears well-defined rows of backwardly directed teeth; the dorsal or upper surface is concave, the concavity being accentuated (except at the tip) by a shallow groove in the mid-line and by dorsally directed extensions of the dorso-lateral margins (figs. 5, 6). Posteriorly, the shallow groove is continuous with a more distinct groove called the hypostomal gutter (figs. 7, 8).

The *palps* arise one on each side of the hypostome. Each palp consists of four segments or articles; the first or proximal article is flattened along the mesial surface, and lies closely pressed against the side of the hypostome with which it fuses near the basis capituli (figs. 1, 6–9). The palps are longer than

the hypostome, which extends to the level of the third palpal article.

The chelicerae. Each chelicera consists of a long subcylindrical shaft, the proximal half of which is expanded to form a bulbous base lying within the cavity of the basis capituli and projecting into the body cavity. Each shaft is slightly flattened along the mesial surface (figs. 3-15). The distal half of the shaft bears a ventro-medial flange concave towards the mid-line; and this flange, together with its counterpart on the other shaft, encloses a tubular space when the shafts are closely opposed to each other (fig. 4). Distally, a small pyramidal digit, bearing laterally directed teeth or cusps, articulates with each shaft. membranous covering, known as the hood, surrounds the digit, leaving only the points of the cusps exposed. The hood in O. moubata has its origin on the cheliceral shaft (figs. 1-3). As in other ticks, the digit can be made to oscillate in a mediolateral direction by the action of flexor and extensor muscles, which originate in and occupy the bulbous base of the shaft. The muscles, on contraction, actuate the digit by means of tendons which lie for the greater part of their length within tubular channels in the wall of the shaft. The muscles and tendons are not figured in the illustrations. When a cheliceral shaft is fully retracted, the digit is then dorsal to the tip of the hypostome (fig. 3).

The cheliceral sheaths. These are derived from the dorsal conical prolongation of the basis capituli. In O. moubata the outer and inner sheaths described by Robinson and Davidson (1913a) are recognizable, but there is, in addition, a third paired sheathing structure, each part of which we refer to as

a cone sheath.

In order to facilitate an understanding of the three sheathing arrangements, it is desirable to stress here that what is called the outer sheath is, in fact, the exoskeleton of the dorsal conical prolongation of the basis capituli, and that these terms are synonymous. This is clearly seen to be the case in fig. 8. Generally, in this paper, the term 'outer sheath' is used when the sheaths of the chelicerae are being discussed, whereas the phrase 'dorsal conical prolongation of the basis capituli,' or a modification of it, is employed when the prolongation is being considered as a part of the capitulum and not with special reference to its relationship with the chelicerae. In certain of the illustrations of transverse sections, the contractions for both terms are given together, e.g., fig. 8, D.C.P. (S.CH.), but this method is restricted arbitrarily to that part of the dorsal surface of the unpaired region of the prolongation which lies close to the inner sheath (figs. 7, 8). Sections anterior to this region bear the symbol S.CH. (figs. 5, 6), while for those posteriorly situated the contraction D.C.P. is used (figs. 9-12). Since the ventral surface of the dorsal conical prolongation is always closely associated with the chelicerae, it is, when named in the figures, denoted as the outer sheath (S.CH.).

The *outer* sheath. The truncated apex of the dorsal conical prolongation of the basis capituli is bifurcated, and one cheliceral shaft passes into each fork. The cavity of the prolongation does not extend into the forks, and each shaft is therefore, at this level, ensheathed by the exoskeletal wall of the prolongation (figs. 3, 5, 6). The sheath thus formed round each shaft constitutes the beginning of the outer sheath, which is therefore a paired structure distally. The paired outer sheaths fuse together, at first dorsally, to form a stout chitinous ridge which marks the anterior limit of the cavity of the dorsal conical prolongation of the basis capituli. The origin of the ridge is indicated by the arrows in fig. 6. From this ridge, the outer sheath sweeps dorsally away from the chelicerae to form the outer visible surface of the dorsal conical prolongation of the basis capituli (figs. 2, 3, 7–12).

At the point of fusion of the paired outer sheaths, the ventral surface of the united forks is at first closely moulded to the contour of the cheliceral shafts (fig. 7), but in later sections the ventral part of the fused outer sheaths tends to flatten out, to form a platform ventral to the chelicerae but slightly arched between them (figs. 8–12). Where the cavity of the dorsal conical prolongation of the basis capituli merges with that of the basis capituli proper, the ventral part of the outer sheath, or, in other words, the ventral surface of the dorsal conical prolongation of the basis capituli, passes into the cavity of the basis capituli as a plate underlying the chelicerae. This plate is known as the subcheliceral plate (figs. 3, 13, 14), and is not continuous laterally with the wall of the basis capituli.

The *inner* sheath. This takes the form of a membranous lamina arising from the stout chitinous ridge dorsal to the chelicerae (fig. 3) and extending posteriorly into the cavity of the dorsal conical prolongation of the basis

It remains in close association with the cheliceral shafts and continues as a membrane above them, eventually fusing, as described below, with their walls at the proximal end of the bulbous bases. There is no discrete inner sheath ventral to the chelicerae (figs. 7–14). In the anterior region of the subcheliceral plate, a vertical septum appears between the chelicerae. The septum extends from the plate to the inner sheath, thus separating the bulbous bases of the shafts (figs. 11-13). The shafts extend further into the body cavity than the subcheliceral plate, and at the posterior end of the plate each shaft, as a result of a vertical division of the septum, lies free from its neighbour and is surrounded only by a sheath with which it fuses proximally (figs. 3, 14, 15). At this level the sheath may be considered as being derived partly from the inner sheath and partly from the subcheliceral plate. From the sections it will be seen that, within the dorsal conical prolongation of the basis capituli, the inner sheath is continuous laterally with the outer sheath, and that, within the basis capituli, the inner sheath fuses on each side with the subcheliceral plate. Thus, the chelicerae are enclosed in a wide tubular cavity. Posteriorly, the tube is divided into two parts by the septum and finally by the vertical fission of the septum. The independence of the chelicerae posteriorly is, of course, essential for the alternate protrusion of the shafts which occurs during the penetration of tissues.

The *cone* sheaths. Each cone sheath arises as an inflection backwards of a thin membranous layer from the distal margin of the forks of the outer sheath. The membranes pass into the cavity containing the chelicerae, and each fuses with a cheliceral shaft at a point near its bulbous base. Each chelicera is related to its cone sheath in a manner similar to that of a rod passing through the apex of a cone (figs. 2, 3, 5–10). Since each sheath fuses with a cheliceral shaft, it follows that the protrusion of a chelicera necessitates an evagination of its cone sheath; this has been ascertained from sections and the manipulation of whole capituli. These cone sheaths would appear to serve a useful function in preventing tissue débris and blood from gaining access to the deep recesses which extend to the bases of the chelicerae.

Christophers (1906) states that the chelicerae lie surrounded by a loose sheath of a synovial nature. Robinson and Davidson (1913a) describe an inner and outer sheath in Argas persicus, but the inner sheath is paired, each part completely encircling a shaft throughout its length and arising from the distal extremity of a fork of the outer sheath, in the same way as the cone sheaths in O. moubata. True (1932) describes for O. coriaceus a sheathing arrangement similar to that of A. persicus. It is interesting to note that Nuttall, Cooper and Robinson (1908) give an account of sheaths in Haemaphysalis punctata C. and F. which are similar to the cone sheaths in O. moubata. In this Ixodid tick, the forks of the outer sheath are long and extend to the cheliceral digits. The sheaths, similar to the cone sheaths, join the shafts near the articulation of the digit. The authors state that these membranous sheaths serve to draw

the paired outer sheaths over the digits, thus protecting them when the chelicerae are fully retracted. The 'shagreened sheath' is clearly described in the same work. It is formed by the presence of backwardly directed spines on the dorsal surface of the forks of the outer sheath. The forks in *O. moubata* are very short and do not carry spines.

The buccal canal and the buccal cavity. From an examination of sections it is seen that the fissure between the dorsal and the ventral components of the mouth-parts takes the form of a tubular space, which, however, becomes progressively more compressed in a dorso-ventral direction towards the origin of the mouth-parts in the basis capituli (figs. 3-10). This incomplete tube is called the buccal canal. Distally, the canal is formed by the close apposition of the flanged chelicerae supported by the slightly concave solid hypostome (fig. 4). In the region of the apex of the cheliceral sheaths the flange on the shafts becomes indistinct, but the tubular nature of the canal is maintained by the modified contour of the ensheathed shafts and the deepening concavity of the hypostome (figs. 5, 6). In subsequent sections through the dorsal conical prolongation of the basis capituli, the canal is seen to be embraced, dorsally by the medially arched ventral surface of the prolongation, ventrally by the grooved concave upper surface of the hypostome, and laterally by the extensions of the dorso-lateral margins of the hypostome together with the closely applied palps (figs. 7, 8).

Certain of the sections (figs. 6-8) show also the presence of a tongue-like process in the centre of the buccal canal. It is important to note that the tonguelike process is a hollow organ and that it becomes progressively broader towards its point of origin on the dorsal surface of the hypostome. Where the tonguelike process fuses laterally with the hypostome (fig. 9) the hypostomal gutter is closed dorsally, thus forming the opening into the pharynx. The opening is referred to as the pharyngeal orifice. Examination of sections at this level leads to the conclusion that, although the buccal canal may here be functionally a complete tube, morphological union has not yet occurred between the fused hypostome and palps, and the ventro-lateral margin of the dorsal conical prolongation of the basis capituli. According to Nuttall, Cooper and Smedley (1905), Robinson and Davidson (1913a), and True (1932), fusion of these parts laterally marks the beginning of the buccal cavity in ticks. Therefore, it must be stated that in O. moubata the pharynx opens into the buccal canal, and not through the floor of the buccal cavity, as is the case in other Argasid and Ixodid ticks. In O. moubata, the buccal cavity is formed a short distance posteriorly to the opening of the pharynx (fig. 11) and, as in other ticks, forms a blindly ending pouch, which, however, receives a salivary duct at each postero-lateral angle of the pouch (figs. 3, 12). The floor of the buccal cavity is continuous with the dorsal surface of the hollow tongue-like process, while its roof is formed by the posterior limit of the ventral surface of the dorsal conical prolongation of the basis capituli. It may be noted here that chitinous thickenings, derived

from the walls of the buccal cavity, strengthen the subcheliceral plate on each side. The subcheliceral plate and the thick chitinous walls in the region of the buccal cavity and the pharynx, and between the palps and the hypostome basally, constitute the 'endoschlerites' mentioned by Christophers (1906).

That the pharynx does not open into the buccal cavity is an anomalous conclusion, but this arises only from the adoption of a nomenclature which, in this instance, creates the anomaly. Christophers (1906) employs the term 'mouth' to denote the entire space enclosed by the mouth-parts, and Patton and Cragg (1913) refer to the buccal canal and buccal cavity together as the buccal cavity. A reconsideration of the limits of the true buccal cavity in ticks would appear to warrant attention, but, provisionally, the definition of Robinson and Davidson (1913a) is accepted.

The tongue-like process. This is a part of the buccal apparatus of ticks which has not been fully described by previous authors, or which has been the subject of descriptions and interpretations differing from those given below. In particular, the present account of the tongue-like process does not agree with Sen's description of the mouth-parts of ticks. More detailed considerations of these points follow the description below of the capitulum of O. tholozani.

The tongue-like process is a somewhat triangular hollow flap (figs. 3, 16) arising from a broad base on the dorsal surface of the hypostome. The flap extends almost to the level of the apex of the dorsal conical prolongation of the basis capituli, and in both sections and dissections it appears to be closed at the anterior end. The dorsal wall of the tongue-like process is thin and membranous and is frequently wrinkled in sectional view (figs. 3, 7). Near the base of the process the dorsal wall is thickened laterally, leaving only a thin mesial membrane (fig. 8). The chitinous thickenings fuse in the mid-line, ventral to the mesial membrane, to form a stout transverse bar which passes back to contribute to the formation of the floor of the buccal cavity and the roof of the pharynx (figs. 8–12). The mesial membrane persists for a short distance within the buccal cavity to form a double floor, but becomes fused with the true floor of the cavity posteriorly (figs. 3, 11, 12), i.e., a dorsally thin-walled pouch of the tongue-like process projects into the buccal cavity.

The ventral wall of the tongue-like process is thin and membranous, except for a mesial rod-like thickening, which does not, however, pass to the tip of the process (figs. 3, 6–8). It is of particular interest to note that, where the tongue-like process fuses laterally with the hypostome to form the pharyngeal orifice (fig. 9), the ventral wall of the process appears as a horizontal septum stretching across the orifice. The septum continues for some distance into the dilatable portion of the pharynx, within which it lies close to the dorsal pharyngeal wall and remains attached laterally to the mesial wall of the short dorso-lateral folds of the pharynx (figs. 3, 10–13). The septum remains patent within the first third of the dilatable part of the pharynx, but thereafter it becomes fused with the pharyngeal wall (fig. 3). The tongue-like process is therefore the

anterior part of a thin-walled closed chamber, the posterior part of which lies in the dorsal region of the anterior third of the pharynx.

The rod-like thickening in the ventral wall of the tongue-like process forms a short vertical septum in the lumen of the chamber (figs. 3, 10), and it is convenient to consider this septum as marking the point where the posterior part of the closed chamber is in communication with the anterior part, or, in other words, with the cavity of the tongue-like process.

No cellular elements have been detected within the chamber, nor has the nature of its contents been satisfactorily determined. It can be stated, however, that its walls are of a chitinous nature and resist the action of caustic soda. In two of the capituli sectioned after rapid fixation in hot 70 per cent. alcohol, the lumen of the chamber showed finely granular particles grouped in a loose reticulum. These capituli had been bathed in the contents of the ticks' gut diverticuli during dissection, and, in the same sections, a similar reticulum, together with blood corpuscles, was found interspersed amongst muscle bundles in the basis capituli. Blood corpuscles have not been observed in the cavity of the chamber. It is considered possible that the reticulum in the chamber had resulted from the entry of gut-contents into the chamber, due to injury of its walls during dissection. In all other sections of capituli, the contents of the chamber are apparently structureless. No coloured fluid was discernible in tongue-like processes exposed by micro-dissection in ticks which had just completed a blood meal on a rabbit. It is presumed that in life the cavity of the chamber contains a colourless fluid.

THE CAPITULUM OF O. THOLOZANI

The capitulum of *O. tholozani* is similar to that of *O. moubata*. The sheaths surrounding the chelicerae consist of the outer, the inner, and the cone sheaths. A closed chamber incorporating a tongue-like process is present in association with the pharynx. The cavity of the chamber, however, extends to a point about half-way along the pharynx (fig. 20), and the anterior part of the chamber, i.e., the so-called tongue-like process, is, relative to the other mouth-parts, longer than its counterpart in *O. moubata* (cf. figs. 16, 17). The capacity of the tongue-like process is greater in *O. tholozani* than in *O. moubata*. As in the latter, the dorsal wall of the process is supported by two lateral chitinous thickenings, while the ventral wall is strengthened by a mesial rod (figs. 17, 19).

THE TONGUE-LIKE PROCESS AND THE STYLET (ORGAN) OF SEN

In both Ixodid and Argasid ticks, previous authors have noted that the floor of the buccal cavity extends over the pharyngeal orifice either as an elastic membrane (Samson, 1909) or as a tongue-like process which may contain a cavity continuous with that of the basis capituli (Robinson and Davidson, 1913a, b; True, 1932). Ruser (1933) elaborates the description by Samson to include a musculature which pulls the tongue-like process towards the pharyngeal orifice. According to these authors, the tongue-like process or the elastic membrane serves to prevent salivary fluid from passing directly into the pharynx.

The works of Sen, however, offer an interpretation of the mouth-parts of ticks differing fundamentally from earlier descriptions, and we propose to direct attention to this interesting subject. It appears to the present writer that the tongue-like process which is described in detail in this paper for O. moubata, and which is also identified in O. tholozani, is the same structure as that referred to as the 'stylet' by Sen (1935b) in O. tholozani. Later (1937) he refers to the 'stylet' as the 'organ.' Sen also observed the stylet (organ) in dissections of a number of specimens representative of Ixodid genera. In 1935b he writes that in ticks 'the sucking apparatus is a stylet which is a tubular continuation of the pharynx,' and, according to his description, this stylet has a 'distinct distal orifice.' He draws attention to the fact that, in one specimen of O. tholozani recently fed on a fowl, blood detritus was found scattered over the inner surface of the wall of the stylet, the buccal chamber (the broad basal attachment of the stylet to the pharynx) and the pharynx, and he considers this as conclusive evidence of his interpretation of the function of the stylet. It must be stated, however, that Sen (1937) appears to entertain a certain degree of doubt concerning the functional significance of the stylet (organ), since he writes: 'The possibility that it is along the lumen of the organ that blood is sucked in is suggested by the fact that in all the dissections so far carried out the organ appeared to be a continuation of the buccal chamber, whilst in one instance, at least, blood particles were observed inside the organ, this particular specimen being one of O. papillipes that had been recently fed on a fowl. On the other hand, the lumen of the organ appeared to be far too narrow to admit of the entry of more than a very limited number of blood corpuscles of normal size at a time.' Furthermore, in his communication of 1937, while discussing the organ in Ixodid ticks, Sen says that it 'terminates distally in what appears to be a distinct orifice, the rim of which may not be in the same plane along the whole circumference.'

THE MECHANISM OF FEEDING IN O. MOUBATA

We are not concerned with the actions involved nor the musculature required in the penetration of the host's tissues by the tick. The reader is referred to Robinson and Davidson (1913a, 1914) for a detailed account of the mechanism actuating the chelicerae. It is well, however, to note that, preparatory to sucking blood, a tick (O. moubata) may insert the chelicerae and hypostome into the host's skin up to the level of the apex of the dorsal conical

prolongation of the basis capituli. The palps do not enter the wound. We propose to consider only the chitinous parts and the musculature concerned in controlling the passage of blood from the wound to the oesophagus.

I. THE MUSCULATURE AND ACTION OF THE PHARYNX

The form and the musculature of the pharynx are arranged on the same plan as has been described for A. persicus, O. savignyi and O. coriaceus by Robinson and Davidson, Christophers, and True respectively. The fusiform shape is shown in fig. 16, and in transverse sections (figs. 13, 14) the muscles acting along the greater part of its length are shown semi-diagrammatically. Dilatation of the pharynx results from the contraction of the dorsal dilator muscles originating on the subcheliceral plate, and of the lateral and ventral dilator muscles radiating to the wall of the basis capituli, or anteriorly (fig. 9) to the stout walls between the palps and the hypostome. Muscles crossing between the apices of the folds of the pharyngeal wall form bands round the pharynx, alternating in distribution with the dilator muscles (fig. 3). Contraction of these muscle bands causes constriction of the pharynx. The negative pressure created within the pharynx as it dilates sucks blood from the wound into A valve, referred to by Robinson and Davidson and by Christophers as the 'proventricular fold,' prevents the contents of the gut diverticuli from being sucked towards the pharynx when it is dilating. When the pharvnx begins to contract, the blood passes into the narrow tubular oesophagus attached to the proximal end of the pharynx, and does not apparently regurgitate into the wound. Regurgitation is probably prevented, as we shall see, by a mechanism involving the closed chamber and its anterior part, namely, the tongue-like process.

It has been noted that in *O. moubata* there are, in addition to the large dilator muscles originating on the subcheliceral plate, a number of short muscles running from the floor of the buccal cavity to the dorsal wall of the pharynx. Most of these short muscles are practically vertical in direction, but two strands run from an anterior position on the buccal cavity floor to a more posterior attachment on the pharynx wall (figs. 3, 11, 12).

II. THE ACTION OF THE CLOSED CHAMBER AND TONGUE-LIKE PROCESS

It seems probable that, as the pharynx dilates, the effect of the negative pressure in the pharyngeal lumen, together with the pull of the short and long dorsal dilator muscles upon the dorsal pharyngeal wall in the region of the posterior part of the closed chamber (fig. 13), would be to increase the capacity of the chamber posteriorly. Conversely, relaxation of the dilator muscles, together with the contraction of the constrictors of the pharynx, would reduce the capacity of the chamber within the pharynx, owing partly to the depression

of the dorsal pharyngeal wall and partly to the pressure of the blood being compressed by the constricting pharynx. It is considered probable that, when the capacity of the posterior part of the chamber is reduced during the constriction of the pharynx, the contents of the chamber, presumably fluid, are pressed into the cavity of the tongue-like process, so that its thin membranous walls become distended. On the other hand, when the pharynx dilates, the fluid within the tongue-like process is sucked back into the posterior part of the chamber, with the result that the tongue-like process collapses. The vertical septum derived from the rod-like support of the ventral wall of the tongue-like process maintains a communication between the cavity of the tongue-like process and the posterior part of the chamber.

III. THE MUSCULATURE AND ACTION OF THE HYPOSTOME

Muscles similar to the ventral dilator muscles of the pharynx are present in the hypostome, and extend from the lateral and ventral walls of the hypostome to the walls of the hypostomal gutter and to points on each side of it (figs. 7, 8). This musculature does not occur beyond the point figured (fig. 7).

The general view regarding the passage of blood to the pharvnx is that it is drawn, by dilatation of the pharynx, up the buccal canal, along the hypostomal gutter, and thence into the pharyngeal orifice. With that view we agree in the main, but, in the transverse sections of one capitulum, it was observed that the concave upper surface of the hypostome was so deeply furrowed that the buccal canal, for a considerable distance, was partially divided into a dorsal channel and a ventral channel (fig. 21). The shallow groove described in the preliminary account of the hypostome forms the floor of the ventral channel or furrow. The dorsal channel is that part of the buccal canal between the cheliceral sheath and the hypostome, excluding the furrow. It will be observed from the figure that the tongue-like process occupies a central position in the dorsal channel, but it is probable that, during sucking, it lies close to the furrow and covers the slit-like communication between the furrow and the dorsal channel. The furrow, or ventral channel, is continuous with the hypostomal gutter leading to the pharynx, and appears to be the true food channel, whereas the dorsal channel, above the tongue-like process, is in line with the buccal cavity and the salivary ducts and may be considered as a salivary channel. It is possible that the salivary secretion is conducted via this channel and the tube formed by the flanged chelicerae to the wound, but, from a study of all the sections made, it seems more probable that the suggested food channel and salivary channel meet in the region of the flanges of the chelicerae, and that the tube so formed marks the level at which salivary secretions mix with the blood. It appears, therefore, that the depth of the hypostomal furrow can be varied, and it is probable that this variation results from the action of the muscles in the base of the hypostome (figs. 7, 8), relaxation of these closing the hypostomal gutter

and almost obliterating the hypostomal furrow anterior to the gutter. Conversely, contraction of the muscles would alter the gutter from a closed cleft to a V-shaped groove, and, at the same time, create the deep furrow anteriorly. Of course, such a suggestion presupposes a certain degree of flexibility in the walls of the hypostome. The dorso-lateral extensions and the dorsal surface of the hypostome are, in parts, thinner than the ventral and lateral walls, an observed fact which certainly supports the opinion that the dorsal surface of the hypostome is subject to variation in configuration (cf. figs. 5, 21).

It is suggested then that, during the sucking phase of the pharynx, blood passes from the wound into the buccal canal formed by the flanged chelicerae and the hypostome, but that it tends to be diverted into a deep furrow in the hypostome, the furrow being formed by the contraction of muscles acting upon the hypostomal gutter and the thin dorsal wall of the hypostome on each side of it. In the region of the cheliceral sheaths the deep furrow or food channel is further cut off from the dorsal or salivary channel by the tongue-like process.

IV. The Mechanism for Preventing the Regurgitation of Blood into the Wound

As the pharvnx becomes gorged with blood, it is assumed that the dorsal dilator muscles increase the capacity of the posterior region of the chamber, so that the contents of the chamber are concentrated in the posterior region, leaving the tongue-like process in a collapsed condition and over-lying the food channel in the hypostome. When fully dilated the pharynx must constrict in order to propel its contents into the oesophagus. Such a force, as we have already suggested, must tend to drive the blood back into the wound, but it seems probable that the following two factors operate to prevent regurgitation. Firstly, the relaxation of the muscles in the hypostome converts the V-shaped gutter into a closed cleft and obliterates the furrow associated with it; secondly, the contents of the closed chamber are forced forwards into the tongue-like process and distend it until the ventral surface lies against the upper surface of the hypostome, the rod-like thickening being pressed against the closed cleft. Thus the distension of the tongue-like process and the closure of the hypostomal gutter completely block the passage from the pharynx to the buccal canal during the constrictive phase of the pharvnx.

The two extreme conditions suggested in this conception of the sucking apparatus are represented diagrammatically in fig. 22, A and B. The diagrams also illustrate certain points concerned in a possible mechanism for the ejection of salivary fluid, a subject which is discussed below.

It has been stated that the anterior narrow portion of the pharynx may serve to prevent regurgitation of blood into the wound. True (1932) does not consider the problem, and Sen (1935b) associates himself with the conclusion of Robinson and Davidson (1913b), who state that 'The lumen of the organ

[pharynx] . . . is constricted [at the anterior end] . . . , but nothing of the nature of a valve can be recognised. It would, therefore, appear that the vis-a-tergo of the blood in the buccal canal and buccal cavity is greater than that of the contents of the oesophagus and stomach, so that when the pharynx contracts, its contents follow the direction of least resistance, i.e. through the oesophageal orifice.'

V. A Possible Mechanism for the Ejection of Salivary Fluid

The structural details of the tongue-like process suggest that it may contribute to the formation of a sucking and pumping mechanism for the ejection of the salivary fluid. Sections indicate that, in life, the dorsal conical prolongation of the basis capituli lies closely pressed against the dorsal surface of the fused hypostome and palps, so that the anterior opening to the buccal cavity is restricted to a slotlike opening above the mesial membrane of the tongue-like process (figs. 9, 10). It is suggested that, during the constrictive period of the pharynx, the mesial membrane of the tongue-like process is distended to close the buccal cavity anteriorly; consequently, the buccal cavity, the salivary ducts and the salivary glands form a closed system. The two ducts connect the buccal cavity with the alveoli of the glands which lie within the body cavity immediately posterior to the basis capituli, and each duct shows a spiral-like thickening which lies between the chitinous lining of the duct and the investing epithelial layer. The presence of these spiral-like ridges has led to comparisons of the ducts with tracheal tubes. Nordenskiöld (1908) points to the relationship of the ridges to the chitinous lining and the epithelial layer; Robinson and Davidson (1913b) and True (1932), while also recognizing this point, note that, since the thickenings persist after treatment with caustic potash, they may therefore be considered as part of the chitinous lining. A duct in section is sometimes comma-shaped, or, in other instances, almost circular in outline. Presupposing that the duct walls react to increasing pressures in a manner analogous to the tracheal tubes in some insects (Wigglesworth, 1934), it is suggested that during dilatation of the pharvnx the ducts would be compressed and their lumen reduced or obliterated. versely, constriction of the pharynx would, by reducing the pressure transmitted to the duct walls by the haemocoele fluid, cause them to assume an almost circular The lumen of each duct, therefore, would increase as the pharynx constricted, and, since the buccal cavity would be closed anteriorly in this phase, salivary fluid would be drawn from the alveoli of the glands into the lumen of the ducts. When the pharynx begins to dilate, the first result would be the collapse of the tongue-like process, and consequently the slot-like opening above the mesial membrane would become patent. Thus, a communication is established between the closed salivary system and the salivary channel of the buccal canal. The patency of the aperture would coincide with the commencement of the suggested compression of the salivary ducts caused by the dilatation of the

pharynx within the relatively confined space of the cavity of the basis capituli, and salivary fluid would therefore be expressed from the ducts into the buccal canal. Presumably the ejection of the salivary fluid would be facilitated by a small negative pressure created in the salivary channel as a result of the collapse of the tongue-like process. It has been noted earlier that short dorsal dilator muscles of the pharynx are attached to the floor of the buccal cavity, and it seems probable that, since the floor is thin, contraction of these dilator muscles may depress the floor as well as dilate that part of the pharynx with which they are concerned. In this way, depression of the floor of the buccal cavity would further assist the discharge of salivary fluid from the ducts to the wound. Thus, the collapse of the tongue-like process and the depression of the floor of the buccal cavity, together with the compression of the salivary ducts, form respectively a sucking and pumping mechanism designed to discharge salivary fluid into the distal region of the buccal canal during the sucking phase of the pharynx.

THE HOMOLOGY AND ANALOGY OF THE TONGUE-LIKE PROCESS

It is interesting to note that in a Gamasid mite (Laelaps echidninus Berl.) described by Stanley (1931) the floor of the buccal canal is formed by the hypostome, but that on each side the canal is closed by two interlocking processes which Stanley refers to as the stylus and the maxilla. The maxilla is the more ventral process. They occupy a position comparable with that of the extension of the dorso-lateral margin of the hypostome in ticks, and extend almost to the level of the tip of the hypostome. Each maxilla is continuous posteriorly with the ventro-lateral wall of the pharynx, but each stylus arises as a process from the mesial surface of the basal portion of a palp. The palps occupy positions similar to those in ticks, and the roof of the buccal canal is formed by the chelicerae and their sheaths. It is important to note that in Laelaps the salivary ducts are paired, and that each duct passes along the cavity of the basal part of a palp, but penetrates the mesial surface of the palp to enter the stylus, within which it continues as a tube to open distally at the tip. Further, there are two other processes in the buccal complex, which are described by Stanley as follows: 'Between the bases of the mandibles (chelicerae), ventral to them, and having their support partially on the thickened anterior ends of the mandible sheaths, and partially on the anterior end of the dorsal wall of the pharynx, are the lanceolate vomer and lingula. . . . The ventral surface of the lingula is continuous with the dorsal wall of the pharynx.' The lingula is ventral to the vomer.

It is reasonable to conclude that the tongue-like process in ticks is the homologue of the lingula, or of the lingula and vomer fused, of *Laelaps*, and that the close association of the tongue-like process in ticks with the openings of the salivary ducts is a secondary feature resulting from the atrophy or suppression of the styli.

Regarding the suggestion by Sen (1934a, b, 1935b, 1937) that the organ (stylet) in ticks may represent the homologue of the hypopharynx in bloodsucking Diptera, it appears to the present writer that any attempt to homologize the tongue-like process in ticks with mouth-part units in blood-sucking insects must await a satisfactory conclusion concerning its homologies in other Acari and, probably, in a wider range of arthropod types. It may be pointed out, however, that Imms (1934, p. 22) writes that the hypopharynx in insects is a ' median tongue-like process arising from the floor of the mouth-cavity, and bearing the aperture of the common salivary duct,' and that Snodgrass (1935, p. 127) defines the hypopharynx as a 'median post-oral lobe of the ventral wall of the gnathal region of the head anterior to the labium.' To correlate these statements with the present description of the tongue-like process in ticks leads again to the problem of what constitutes the true buccal cavity in ticks. is a point upon which we have not expressed an opinion. It is, however, worth noting that the tongue-like process in ticks arises from the roof or dorsal wall of the pharynx. We are not, therefore, prepared to subscribe to the view that the tongue-like process in ticks may be the homologue of the hypopharvnx in insects.

The hypopharynx in insects may contain a tubular cavity opening at the tip of the organ and continuous posteriorly with the common salivary duct. Such is the case in all blood-sucking Diptera except those of the genus *Culicoides* (Jobling, 1935a). In so far as the tongue-like process in ticks may play a part in directing the flow of salivary fluid to the wound, it may be said to be partly analogous to the insect hypopharynx.

In Dr. Sen's first communication (1934a) an hypothesis concerning the rôle of the hypopharynx in 'biting flies' is put forward. Certain results having a bearing upon this hypothesis are briefly reported by Sen (1935a, c), and these are criticized by Jobling (1935a, b). We are not concerned with the particular entomological points arising in that discussion, but it is apparent that the description in the present paper of the tongue-like process in O. moubata and O. tholozani does not give support to Dr. Sen's original hypothesis (1934a) that the discovery of the stylet in ticks, as described by him, 'points to the conclusion that the sucking apparatus in biting Diptera is not formed by the apposition of the labrum-epipharynx and the hypopharynx as has been hitherto supposed, but that it is a tubular extension of the pharynx itself; . . . that the lumen of the sucking stylet in biting flies is, in all probability, what has, till now, been regarded as the hypopharyngeal extension of the salivary duct.'

SUMMARY

1. The structure of the capitulum of O. moubata (M.) and of O. tholozani (L. and M.) is described. It is found that the capitulum is similar in the two species.

2. An account is given of the musculature of the pharynx and of the hypostome in O. moubata, and their action during feeding is discussed.

3. A tongue-like process is described in detail. It is the anterior part of a closed chamber which extends into the pharynx and fuses with its dorsal wall. The tongue-like process would appear to act as a distensible pouch, which, together with the closure of the hypostomal gutter leading to the pharynx, blocks the pharyngeal orifice during contraction of the pharynx, thus preventing the regurgitation of blood into the wound.

4. A possible mechanism for the ejection of salivary fluid is suggested. The tongue-like process plays an essential part in this mechanism.

5. The tongue-like process appears to be the same structure as the stylet (organ) described in ticks by Sen (1935b, 1937).

6. The present writer does not consider the tongue-like process to be the homologue of the hypopharynx in insects. It is apparently the homologue of one or more structures, namely, the lingula, or the lingula and the vomer, in a Gamasid mite.

7. In so far as the tongue-like process may play a part in the discharge of the salivary fluid, it is to that extent analogous to the hypopharynx in insects.

8. The present paper does not support the hypothesis suggested by Sen (1934a) concerning the rôle of the hypopharynx in 'biting flies.'

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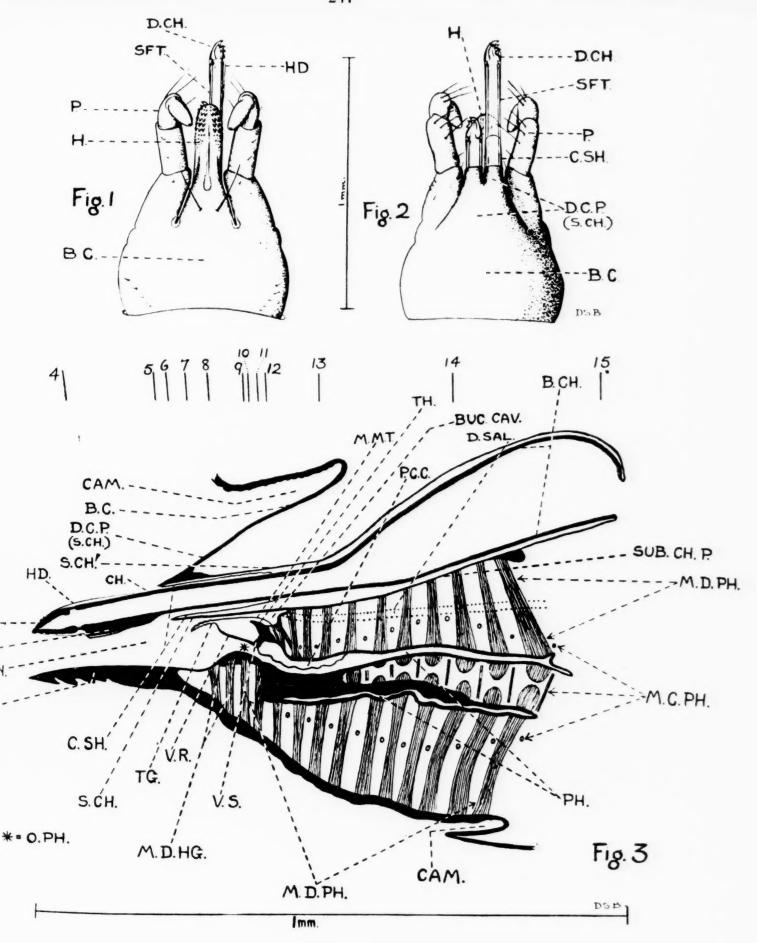
EXPLANATION OF FIGURES 1-3

- Fig. 1. O. moubata, female; capitulum, ventral aspect; left chelicera protruded.
- Fig. 2. O. moubata, female; capitulum, dorsal aspect; right chelicera protruded.
- Fig. 3. O. moubata; capitulum; longitudinal vertical section based upon sections through females and a male. Muscles semi-diagrammatic.

KEY TO THE LETTERING OF ALL FIGURES

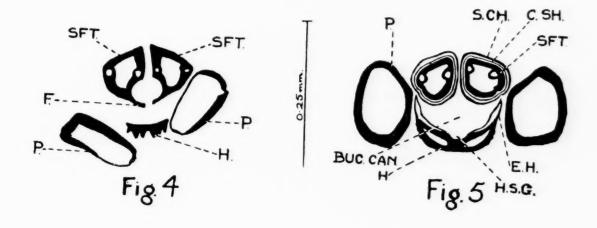
The lines numbered 4 to 15 in fig. 3, and 4 to 14 in fig. 16, show the levels at which the similarly numbered transverse sections are taken. With the exception of figs. 1 and 2, the figs. are based upon camera lucida outlines of actual specimens. Consequently, the levels of the transverse sections indicated in figs. 3 and 16 are approximate.

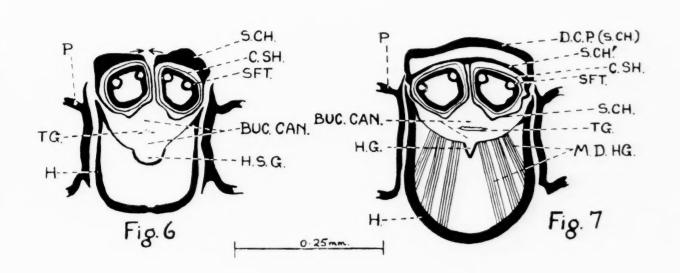
B.C.,	basis capituli.	M. D. HG.,	dilator muscles of hypostomal
	expanded base of chelicera.	,	gutter.
	, buccal canal.	M. D. PH.,	dilator muscles of pharynx.
	, buccal cavity.	M. M. T.,	mesial membrane of tongue-like
CAM.,	camerostome.	,	process.
CH.,	chelicera.	O. PH.,	pharyngeal orifice.
	cone sheath.	P.,	palp.
	dorsal cavity of tongue-like	P. C. C.,	posterior part of closed chamber.
	process.	PH.,	pharynx.
D. CH.,	digit of chelicera.	S.,	septum between chelicerae.
D. C. P.,	dorsal conical prolongation of	S. CH.,	outer sheath.
	basis capituli.	S. CH.',	inner sheath.
D. SAL.,	salivary duct.	SFT.,	shaft of chelicera.
E. H.,	extension of dorso-lateral margin	SUB.CH.P.	, subcheliceral plate.
	of hypostome.	TG.,	tongue-like process.
F.,	flange of chelicera.	TH.,	transverse bar formed by fusion
H.,	hypostome.		of lateral thickenings of tongue-
HD.,	hood.		like process.
H. F.,	furrow of hypostome.	V. R.,	ventral rod of tongue-like pro-
H. G.,	hypostomal gutter.		cess.
H. S. G.,	shallow groove of hypostome.	V. S.,	vertical septum in cavity of
M. C. PH.,	constrictor muscles of pharynx.		closed chamber.

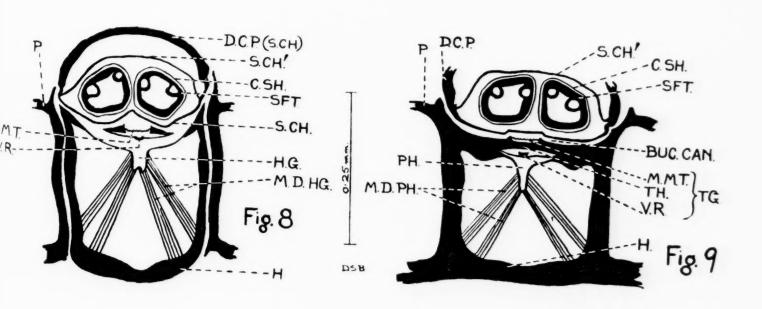


EXPLANATION OF FIGURES 4-9

- Fig. 4. O. moubata, female; capitulum; transverse section through distal half of hypostome. The hood investing the chelicera is omitted from the fig.
- Fig. 5. O. moubata, female; capitulum; transverse section at level of apex of paired outer sheaths.
- Fig. 6. O. moubata, female; capitulum; transverse section at level of paired outer sheaths, showing presence of tongue-like process in buccal canal. The arrows indicate that, in sections between fig. 6 and fig. 7, fusion of the paired outer sheaths occurs.
- Fig. 7. O. moubata, female; capitulum; transverse section posterior to transverse chitinous thickening formed dorsally by fusion of paired outer sheaths. Muscles semi-diagrammatic.
- Fig. 8. O. moubata, female; capitulum; transverse section showing muscles acting on hypostomal gutter; also details of tongue-like process. Muscles semi-diagrammatic.
- Fig. 9. O. moubata female; capitulum; transverse section immediately posterior to the pharyngeal orifice. Note that the buccal cavity is not yet formed. Muscles semi-diagrammatic.

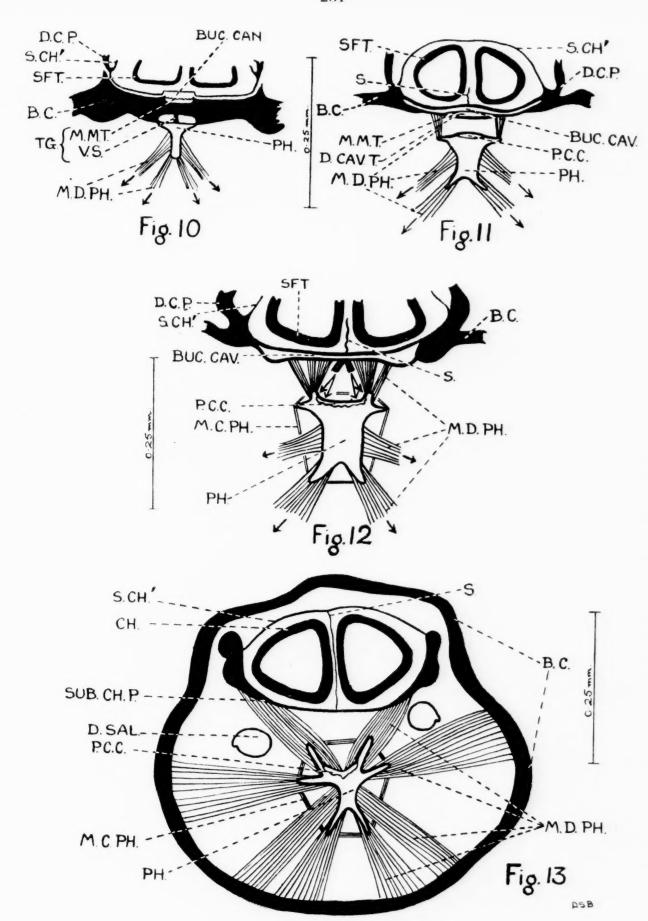






EXPLANATION OF FIGURES 10-13

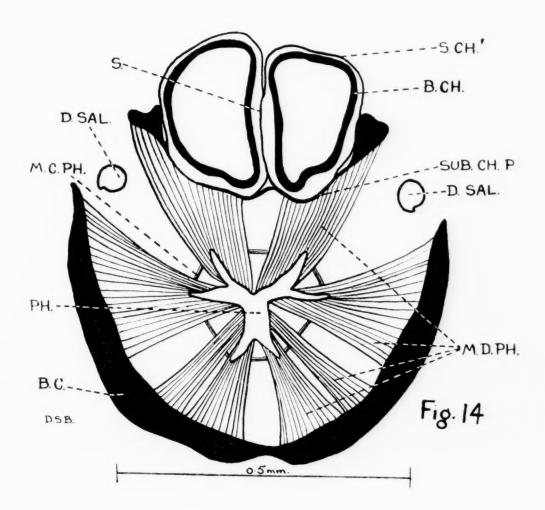
- Fig. 10. O. moubata, female; capitulum; transverse section at level of vertical septum within closed chamber. Muscles semi-diagrammatic.
- Fig. 11. O. moubata, female; capitulum; transverse section through buccal cavity. Muscles semi-diagrammatic.
- Fig. 12. O. moubata, female; capitulum; transverse section at level of short muscles between pharynx and buccal cavity; arrows indicate points of attachment of muscles. Note the lateral widening of the buccal cavity with which the salivary ducts connect. Muscles semi-diagrammatic.
- Fig. 13. O. moubata, female; capitulum; transverse section through basis capituli. Note, particularly, points of attachment of dorsal dilator muscles to pharynx. Muscles semi-diagrammatic.

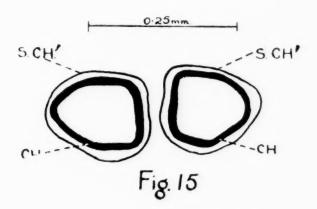


EXPLANATION OF FIGURES 14-15

Fig. 14. O. moubata, female; capitulum; transverse section through posterior limit of basis capituli. Muscles semi-diagrammatic.

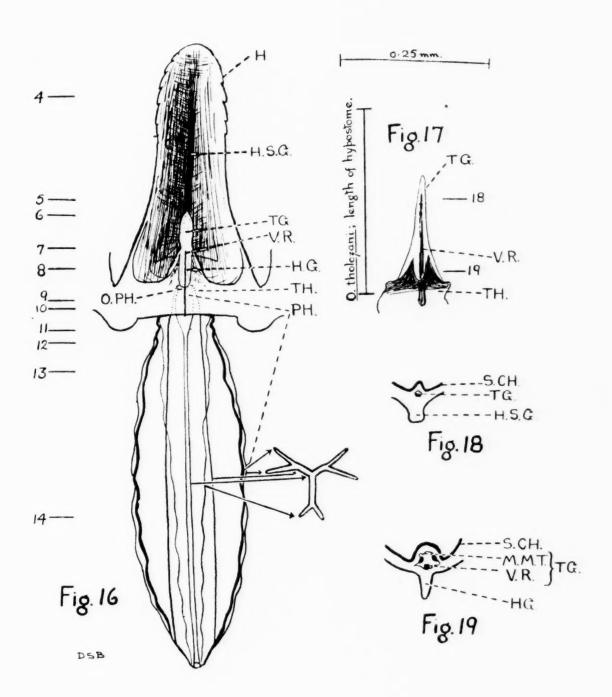
Fig. 15. O. moubata, female; transverse section through proximal ends of chelicerae.





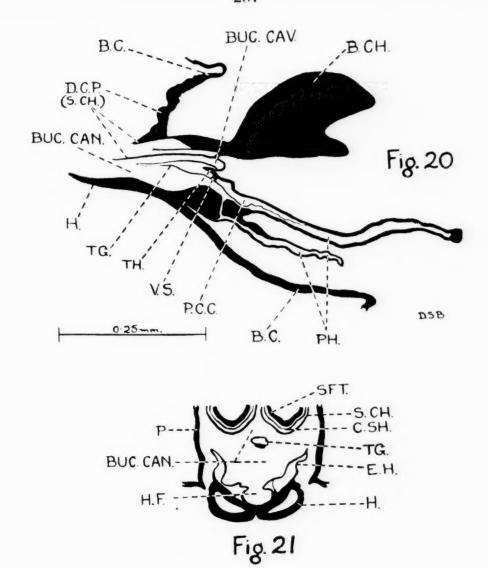
EXPLANATION OF FIGURES 16-19

- Fig. 16. O. moubata, emale; dissection of hypostome and pharynx together, showing tongue-like process overlying hypostomal gutter and pharyngeal orifice (indicated by dotted lines): from a semi-transparent preparation in glycerine jelly. Limits of posterior part of closed chamber not visible. The longitudinal lines of the dissected pharynx are correlated with a transverse section.
- Fig. 17. O. tholozani, female; tongue-like process dissected from capitulum. The length of the process relative to the hypostome in the same specimen is indicated. The lines numbered 18 and 19 indicate approximately the levels of the transverse sections shown in figs. 18 and 19.
- Fig. 18. O. tholozani, female; capitulum; part of transverse section through buccal canal.
- Fig. 19. O. tholozani, female; capitulum; section from same series as in fig. 18, showing details of tongue-like process (cf. O. moubata, fig. 8).



EXPLANATION OF FIGURES 20-22

- Fig. 20. O. tholozani, female; capitulum; longitudinal vertical section to show tongue-like process and posterior part of closed chamber.
- Fig. 21. O. moubata, male; capitulum; transverse section to show deep furrow in hypostome (cf. fig. 5).
- Fig. 22. Diagrammatic representations of the possible effects of the action of the pharynx in ticks during, A, dilatation of the pharynx, and, B, constriction of the pharynx. In A, the arrow above the tongue-like process indicates the direction of the flow of salivary fluid.



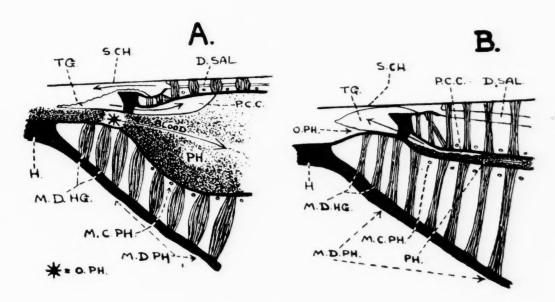


Fig. 22

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A STUDY OF THE BEHAVIOUR OF THE MOUTH-PARTS OF MOSQUITOES WHEN TAKING UP BLOOD FROM LIVING TISSUE; TOGETHER WITH SOME OBSERVATIONS ON THE INGESTION OF MICROFILARIAE

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I. INTRODUCTION

From early times the biting of mosquitoes and the mechanism by which they extract blood from the host have been subjects of interest. More recently this problem, which was previously of more or less academic interest, has become of practical importance, for without knowledge regarding the mechanism of feeding and the injection of salivary secretion we are not in a position to understand the exact processes by which man is infected with the many diseases communicated to him by the bite of the mosquito. To quote an example of pre-eminent importance, information is scanty regarding the precise site in which sporozoites of *Plasmodium* are deposited by the infecting mosquito. Boyd and Kitchen (1939) consider it likely that most of the sporozoites discharged by an infective mosquito at the moment of biting are expelled into the interior of a capillary, but they have shown that, in some cases at least, sporozoites can be demonstrated in the intervascular tissue a few minutes after biting.

Problems of a rather different aspect present themselves in the case of filariasis. Manson (1883), Ashburn and Craig (1907) and O'Connor and Beatty (1937) have drawn attention to the fact that individual mosquitoes appear to take up more microfilariae than can be found in a similar quantity of blood obtained from a finger-prick. Another problem, not so frequently referred to, although illustrated by the figures quoted by the above authors, is that mosquitoes fed on the same patient take up a greatly varying number of microfilariae, apparently independently of their concentration in the peripheral blood. Hinman (1933, 1935) and Galliard (1936) appear to be the only authors who have drawn attention to this fact.

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It is not at present known whether such problems are to be explained by some selective action on the part of the mosquito or whether they are dependent on the particular site or depth of the tissue on which the mosquito is feeding. These and similar lacunae in our knowledge of mosquito-borne diseases prompted us to undertake the experiments to be described.

As already stated, observations on the mechanism by which mosquitoes take up blood extend over a considerable period, but all such previous observations have been limited by the fact that once the fascicle disappeared beneath the skin its subsequent movements could not be observed. Investigations in the past have, therefore, been confined to (1) external observations made on mosquitoes in the act of biting, (2) studies of mosquitoes killed during the act of feeding and subsequently sectioned together with a portion of the tissue on which they were feeding, and (3) purely morphological studies of the mouthparts and their attachments. In the present paper we discuss the results obtained by previous workers along these lines, and compare them with the direct observations which we have made on the behaviour of the mosquito mouth-parts during the act of feeding.

The elaboration of a satisfactory technique for the observation of the behaviour of the mouth-parts of mosquitoes in the frog-web seemed to offer considerable possibilities, since the transparency of the web would render visible the actual movements of the fascicle in the tissue. In addition, frogs are easily obtainable infected with certain species of filaria, and the behaviour of the microfilariae in the capillaries and their ingestion by the mosquito were likely to be capable of observation. Various workers have carried out observations on the behaviour of microfilariae in the capillaries. Lynch (1919) watched the microfilariae of *Dirofilaria immitis* in the exposed omentum of the dog. He, however, regarded his technique as unsatisfactory in that few microfilariae were seen, and under the conditions of exposure the circulation rapidly became abnormal on account of the combined influences of cooling and desiccation. Augustine et al. (1936) have recorded observations on the behaviour of the same species of microfilaria injected into the blood-vessels of bats. They watched the larvae in the capillaries of the bat's wing and noted their ability to progress against the circulation by pressing their coils against the capillary wall. None of these writers attempted to observe the ingestion of these microfilariae by mosquitoes.

II. MATERIAL AND TECHNIQUE

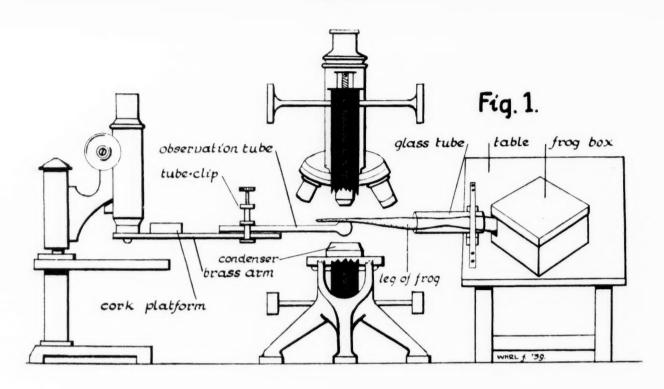
The frogs used were stated by the Englewood Zoological Supply Company of Florida, from whom they were obtained, to be the North American leopard frog, *Rana sphenocephala*. The microfilariae in the circulation are derived from adults in the retroperitoneal space, the worm being *Foleyella dolichoptera* Wehr and Causey, 1939. We are indebted to Dr. Causey, of the Johns Hopkins University, Baltimore, by whose help the frogs used in these experiments were

obtained. Microfilariae were, in severe infections, abundant in the blood-vessels of the web. The number of microfilariae in the venous circulation was estimated by the following method: Blood was obtained from one of the large cutaneous veins which course towards the axilla; these vessels may be exposed by a small skin incision and, when punctured, bleed freely, so that dilution by lymph is reduced to a minimum. The blood was taken up by a white-cell pipette in which it was diluted 1:20 with 3 per cent. acetic acid, which killed the microfilariae in an extended attitude so that they were easily counted in the chamber. The counting chamber used was that designed by Zschucke (1931) for the estimation of helminth eggs in faeces. In light infections the microfilariae were counted over the whole ruled area $(25 \times 15 \text{ mm.})$, in heavy infections over five bands $(15 \times 1 \text{ mm.})$ at regular intervals.

The frogs most suitable were those of the lightest coloration, a profusion of dark-pigment cells in the skin interfering with observation. The third or fourth web of the hind foot was used, as these were the webs which offered the largest area for examination. The frog was confined for the duration of the experiment in a tin box of dimensions $9 \times 9 \times 6$ cm., at one corner of which a hole, its edges protected by rubber tubing, gave exit to one hind limb (figs. 1, 2). The frog was immobilized in the box by firm packing with moist cotton wool, the leg being fixed externally by passing it through a wide horizontal glass tube (figs. 1, 2) in which cotton-wool padding was used to preclude movement. It was found necessary carefully to pad all pressure points, otherwise superficial gangrene of the skin occurred.

The box and tube were supported on an inclined drawing table (figs. 1, 2) so that their height could be easily adjusted by sliding them up or down; they were fixed in appropriate positions by adhesive tape and drawing-pins.

The mosquito under observation was confined in a small bulb blown at the end of a short piece of glass tubing of 9 mm. calibre; a circular window covered by mosquito-netting, on the upper surface of the bulb, allowed it access to the web. The mosquito, introduced at the other end of the tube, was prevented from escaping by a plunger (fig. 3). The tube was carried horizontally on a flat brass arm attached, in place of an objective, to the tube of a microscope stand. It was secured in the required position on this arm by a screw-down tube-clip which, when loosened, allowed easy adjustment (figs. 1, 2). The brass arm also bore a narrow transverse cork platform, to which were pinned tapes attached to clips, the latter holding the toes of the frog (figs. 1, 2). The clips used were ordinary 'Kirbigrip' hairpins, whose tips were protected by tabs of adhesive plaster (fig. 4); these clips, applied longitudinally to the toes, were light and effective. To each was tied about 15 cm. of narrow tape, so that after three or four clips had been attached to the toes the tapes could be easily pinned down on the cork platform in order to stretch the web (fig. 2). A triangular fragment of cover-slip was then applied to the upper surface of the web and the glass observation tube introduced, adjusted and clamped. The speed of circulation



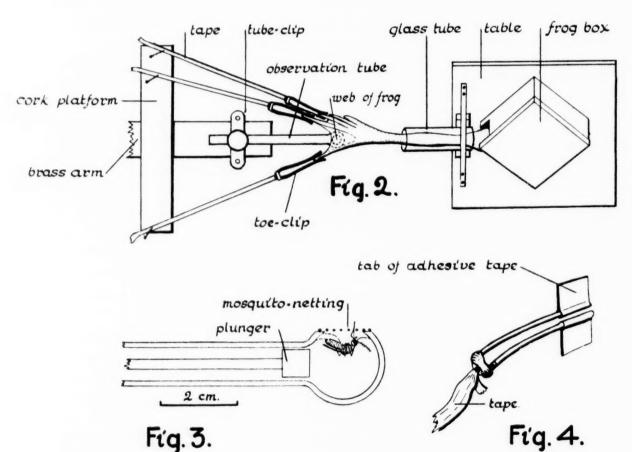


Fig. 1. Elevation of apparatus. The limb of the observation microscope is broken to show the arrangement of the web and observation tube; the tapes and toe-clips are omitted. Fig. 2. Plan of apparatus, to show method of attachment of clips and tapes to stretch the

web.

FIG. 3. Observation tube with mosquito in position for feeding.

Fig. 4. Toe-clip to show attachment of adhesive-tape tab.

in the vessels of the web could be accurately controlled by manipulating the coarse adjustment of the microscope stand, as raising the observation tube caused the web to press more firmly on it, with a consequent slowing of the circulation.

The stage and the top component of the Abbe condenser of the microscope to be used for observation were removed; the condenser was then racked up till immediately below the observation bulb, and the objective focussed on the web (fig. 1). This arrangement had the advantage that the observation microscope was completely independent of the rest of the apparatus and could be moved to centre the appropriate part of the web. The foot of the microscope rested on a glass plate (not shown in the diagram), so that it could be slid about easily. Observation was usually begun with a $1\frac{1}{2}$ inch objective and a $\times 5$ eyepiece and, when the fascicle was located, higher powers (2/3 inch objective, $\times 10$ eyepiece) were substituted; under favourable circumstances the 1/6 inch objective could be used.

Female Aëdes aegypti, which were fed for several days on raisin and then starved for 48 hours before use, were found most satisfactory. About 70 per cent. of such females have been found to gorge when placed with frog in as chamber under a 60 watt lamp. We were unable, however, to induce them to feed directly on the web in the apparatus used, even when stimulated by warm expired air. Recourse had to be made to allowing them to bite the human arm, interrupting feeding after a period of about 30 seconds, and then transferring them immediately to the frog's web. Starved females treated in this way were avid to feed again and frequently inserted their stylets immediately they were brought in contact with the web.

The results which follow apply to experiments carried out with Aëdes aegypti, the strain used having been maintained in this country under laboratory conditions for several years. A few experiments were made with strains of Anopheles maculipennis var. atroparvus and of Culex molestus, but these were abandoned early in the investigation, since it was not found possible to induce them to bite the frog. Although our experiments were carried out with Aëdes, we see no reason to think that their mechanism of biting is likely to differ markedly from that of other mosquitoes; we propose, therefore, to compare our results with those obtained by authors working with different species. Only mosquitoes were used in our investigations, but it is hoped that at a later date the biting habits of other insects may be studied by a similar technique.

About 100 experiments were carried out, during the course of which the process of biting was observed, in whole or in part, on 34 occasions. It would be tedious and of little value to describe these individual experiments, and we therefore propose to combine the results, so as to give a composite picture of the process by which the mosquito obtains blood from the tissues of its host. We have already made reference to three types of previous investigations, viz.: (1) external observations made on mosquitoes in the act of biting; (2) studies

of mosquitoes killed during the act of feeding and subsequently sectioned together with a portion of the tissue on which they were feeding; and (3) purely morphological studies of the mouth-parts and their attachments. Of these latter, by far the most considered statement on the action of the mouth-parts is that of Robinson (1939), when working with *Anopheles maculipennis*. It is as well to mention here that, on the whole, our direct observations have confirmed in a remarkable manner many of Robinson's predictions regarding the functions of the various mouth-parts and their probable behaviour in the tissues. We have, however, been able to show that certain preconceived views regarding the process of feeding in the tissue are incorrect.

III. RESULTS

1. THE MECHANISM OF FEEDING

All observations were carried out on mosquitoes feeding in the inverted position.

The first sign of the mosquito's intention to feed was the repeated application of the tip of the labium to the skin, the palps being often raised during this process. The labella were always kept pressed together, as described by Robinson (1939), and not separated, as has been stated by Vogel (1921) and other authors. The tip of the labium as seen through the web appeared, therefore, as a dark circular patch (fig. 5), from the centre of which the fascicle later emerged. Before the skin was pierced by the fascicle, the labium ceased to wander and remained stationary for several seconds at the selected spot.

As the fascicle penetrated, the labium became increasingly kinked, a process which could only be observed in side elevation (figs. 8, 9). Whether this occurrence was due at first to mechanical sliding up of the labium on the fascicle by the pressure of the skin on its tip, or, as Robinson suggests, to the action of the maxillo-labial muscles, we are unable to state, but we are inclined to think that the latter explanation is correct, since pinching the sides of the head with forceps produces a definite kinking. We never observed kinking of the labium and protrusion of the tip of the fascicle prior to the application of the labella to the skin, as Robinson implies. We have, however, noticed that the labium does not immediately after feeding return to its normal position; it remains bent for several seconds before straightening to enclose the fascicle. This has previously been described by Fülleborn (1908).

As the fascicle was driven deeper into the skin, further bending of the theca took place, and the insect adopted the characteristic attitude shown in text-books, although, as already noted, we have not at any stage of feeding observed divarication of the labella as is sometimes depicted. During the sinking of the fascicle, the palps, kept in a raised position, vibrate, presumably in rhythm with the maxillary movements.

The remainder of our observations refer to the appearance and the behaviour

of the fascicle in the web of the frog. As already described, the labium, looked at with the microscope from above, appeared as a dark circular object which was applied, withdrawn and then re-applied to the skin on numerous occasions. Finally it remained stationary, and at its centre an oscillatory movement could be observed to continue for a few seconds; this movement was presumably due to the cutting of the most superficial layer of the skin by the galeae of the maxillae.

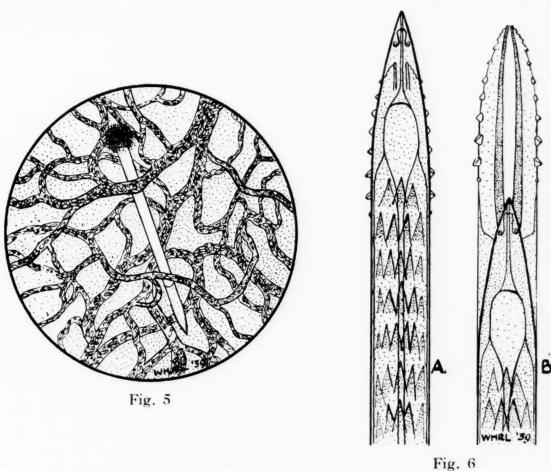


Fig. 5. The appearance of the fascicle of $A\ddot{e}des$ aegypti in the tissues of the frog's web before a blood-supply is tapped. The labral tube is occupied for a short distance at its tip by a column of clear fluid. Proximally the labella are seen as a dark shadow surrounding the point of introduction of the fascicle. (Semi-diagrammatic; \times about 70.)

Fig. 6. A.—The tip of the fascicle of Aëdes aegypti at rest, viewed from above. The galeal teeth are seen protruding beyond the sides of the labrum. B.—Retraction of the labrum in relation to the galeae whose blades remain extended beyond the labral tip and are slightly approximated. (Semi-diagrammatic; × 400.)

The oscillatory cutting movement was followed by the appearance in the circular field of vision of the sharp point of the fascicle. Seen at first in almost end-on view it generally changed its direction to offer a more ventral aspect, in which the details of the labrum and maxillae could be observed. At this stage the labrum was seen as a semi-transparent, brown, chitinous tube, the tip of which had the characteristic 'quill pen' shape (fig. 5) described by Robinson. On each side of the labrum could be seen the rapidly moving galeae of the maxillae.

The tube was worked forward into the tissues with an oscillatory movement, its progress being a series of minute forward thrusts, after the manner of a pneumatic drill. The progress of the labrum forward by such a series of minute jerks supports the idea of Robinson that retraction of the anchored maxillae is responsible for the drawing down of the head and the pulling forward of the labrum—the frequency of oscillation of the latter seemed to be the same as that of the galeae. Although we could see the cutting movements of the galeae clearly, they were so rapid that we were unable to distinguish whether they were synchronous or, as suggested by Robinson, alternate. Alternate movements of the galeae, if they occur, are certainly of small amplitude, as the galeae were never, either singly or together, advanced in front of the tip of the labrum, except that, when the labrum was retracted after a forward thrust, it often did so along the guiding lines of the maxillae, the latter remaining extended, presumably anchored by their teeth (fig. 6, A, B).

Observations on the fascicle in the living tissues never revealed the presence of the mandibles, so that no further reference will be made to them in this paper.

We have already referred to the labrum as a brown chitinous tube, quill-pointed at its extremity. During the piercing of the tissues, before a blood-supply was reached, it could be seen that the major part of the labrum was filled with air, a small portion at its tip being occupied by clear fluid, presumably tissue fluid, which was demarcated from the air by a clear-cut meniscus which sharply defined the diameter (fig. 5). When actual feeding was in progress the tube became less transparent on account of the blood flowing up it, though the speed of flow was usually so great that individual corpuscles could not be recognized.

In all the descriptions which have come to our notice, including that of Robinson, the fascicle is described as a more or less rigid structure which, although it can be inserted in any direction like a hypodermic needle, is yet incapable of any marked bending in its own length. Our observations have shown that, far from being such a rigid structure, it is capable of bending to an extent which enables it to feel its way in any direction. On occasions when the fascicle penetrated the lower surface of the web and continued straight onwards, it passed right through the web. This, however, was obviously an accident, and more often, as soon as the tip of the fascicle, passing between the labella, penetrated the web, it was seen to assume a sharp curve, sometimes almost amounting to a J-form, and to search in the tissue in various directions. This purposive bending involved the distal fifth of the length of the labrum and appeared to be most marked in a dorso-ventral plane (fig. 7). The bending served to guide the subsequent introduction of the fascicle in the required direction as it followed the tunnel bored out in the tissue by the curved cutting tip.

The active bending of the tip of the labrum was seen to be associated with a marked passive flexibility of the remainder of its length, which allowed it to pass through the existing tunnel. This combination of active and passive flexibility was particularly well illustrated when the tip of the fascicle was seen to enter a capillary and subsequently to bend sharply, so that it traversed the

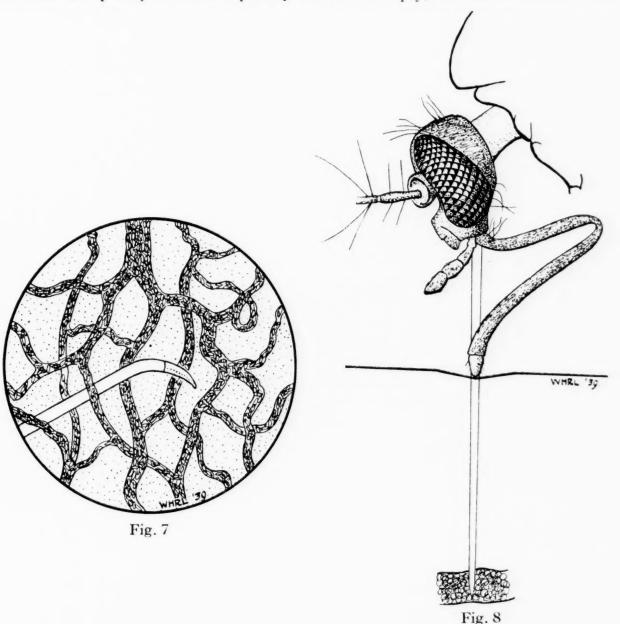


Fig. 7. The fascicle of Aëdes aegypti in the frog's web, showing the active flexibility of its tip. Lateral aspect. (Semi-diagrammatic; × about 70.)

Fig. 8. The generally accepted idea of the behaviour of the fascicle, passing straight into the skin and withdrawing blood from a large capillary in which its tip lies. (Semi-diagrammatic; \times 35.)

lumen of the vessel in a different direction from that in which the more proximal part of the fascicle was lying (fig. 13).

No author refers to this very marked active flexibility at the labral tip, although Robinson mentions the assumption of a slight sigmoid flexure by the unsheathed labrum, presumably under the action of the labral elevator and

retractor muscles. It is possible that this sigmoid curve is an abnormal result of the action of these muscles in the unsheathed labrum, and that when the latter is controlled, at the point of its entrance into the skin, by the labella, all the action of these muscles takes effect at the tip of the labrum, which would explain its marked active flexibility in a sagittal plane.

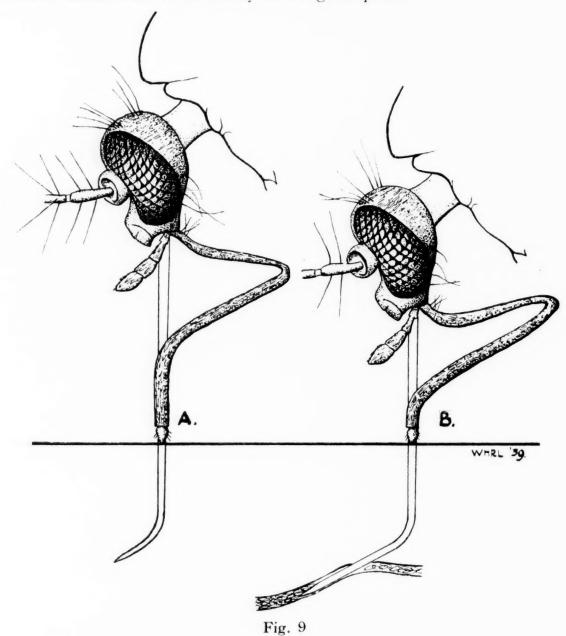


Fig. 9. Stages in the introduction of the fascicle by $A\ddot{e}des$ aegypti as actually observed in the frog's web. A.—The actively flexible tip of the fascicle, having entered the web at right angles to the skin surface, has cut a curved course tending to become parallel to the cuticle. B.—A further stage of penetration. The passively flexible portion of the fascicle has followed the curve previously tunnelled out by the cutting tip, which has penetrated a small capillary and passed along its lumen. (Semi-diagrammatic; \times 35.)

It is more difficult to explain the sideways movement which we have sometimes observed, though it appears possible that considerable lateral direction of the fascicle could be obtained by the action of the labellar extensor muscles, which Robinson depicts as of fairly considerable size.

It will be seen from this account, therefore, that the popular idea of the fascicle as a more or less rigid tube extending straight into the tissues (fig. 8) is a misconception, and that, at any rate in the case of the frog's web, the final course pursued was usually nearly parallel to the surface, although the line of original introduction was almost at right angles (fig. 9, A, B).

The movements of the fascicle after entering the tissue of the frog's web were obviously directed to obtaining blood, but we were unable to satisfy ourselves that the final successful tapping of a capillary by the labrum was anything more than fortuitous, and we failed to find any evidence that 'the terminal sense organs of the labrum serve as directors of the course that the fascicle shall take', as has been suggested by Robinson. In many instances, while probing the tissues, the fascicle of the insect approached very close to a capillary but was not observed to deviate from its course in order to enter it. On other occasions the mouth-parts penetrated through a capillary, apparently without the insect realizing that it had passed through a food-supply.

Usually the fascicle was used like a probe, its direction and depth of entry being constantly altered until a suitable blood-supply was found. Sometimes the supply was obtained almost immediately; at other times probing was carried on for some minutes without success.

It is a common observation that, even when blood is reached, the length of time taken by mosquitoes to engorge varies greatly, and our direct examination under the microscope enables us to advance an explanation. When the fascicle was seen to pierce a capillary and then to be withdrawn, an extravasation of blood into the tissues invariably occurred. This haemorrhage was always sudden and took the form of a characteristic puff of blood, which varied considerably in size. Sometimes the haemorrhage ceased rapidly; on other occasions it continued, so that a considerable pool of blood was formed in the web, sufficiently large to be easily visible to the naked eve. Occasionally the mosquito appeared to be oblivious of the food-supply thus produced and continued probing, but often the tip of the fascicle came to rest in the pool, and the corpuscles could be observed rushing up the tube (figs. 10, 11). In some instances the insect could be seen sucking up the blood as fast as it flowed into the tissues, until it had become completely engorged; on other occasions the haemorrhage was only sufficient to allow of partial satisfaction of its appetite, and further probing of the tissues then took place. This type of feeding on a pool of blood in the tissue, which was kept replenished by a stream of blood from an injured capillary, we propose to refer to as 'pool feeding'. When blood was taken up by this method feeding was always prolonged, the insect taking as long as ten minutes to become engorged, as compared with about three minutes when it fed by the method which we are about to describe as 'capillary feeding'.

In capillary feeding no haemorrhage into the tissues occurred while feeding was in progress. Blood was taken up as a result of the fascicle penetrating into the lumen of a capillary. The usual representation of the fascicle penetrating a large capillary and coming to rest at right angles to it with its tip in the lumen, is a similar misconception to that of considering the fascicle as a more or less rigid structure. It is true that this attitude was occasionally observed, but the normal method of capillary feeding appeared to be that the fascicle, penetrating a capillary at any angle, continued to pass along its lumen. The extent to which the fascicle traversed the capillary varied considerably: sometimes it would pass up the lumen for almost a quarter of its length; more often, however, it only penetrated a short distance, the tip being usually bent just at its entrance (figs. 12, 13).

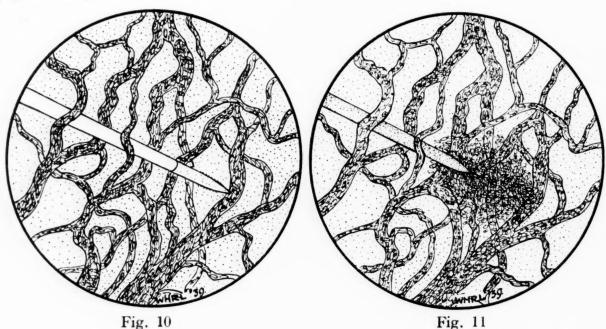


Fig. 10. The fascicle of Aëdes aegypti in the tissues immediately prior to the beginning of 'pool feeding.' The tip of the fascicle, as yet occupied by clear fluid, is about to lacerate a capillary wall.

(Semi-diagrammatic; × about 70.)

Fig. 11. A later stage than that shown in fig. 10: 'pool feeding' in progress. The lacerated capillary is bleeding into the tissue spaces, and the extravasated blood is being sucked up the fascicle, where it is represented by stippling only, as its progress was usually so rapid that individual cells could not be distinguished.

(Semi-diagrammatic; × about 70.)

It has already been stated that in the course of about 100 experiments the mechanism of biting was observed in whole or part on 34 occasions. In some of these mixed feeding occurred, i.e., partially pool and partially capillary; in others probably only one type of feeding took place, but of the latter on only ten occasions was the fascicle kept so clearly in view during the entire experiment that it could be stated with certainty that only one type of feeding took place. On three of these occasions pool feeding occurred and on seven capillary feeding.

The commencement of the capillary type of feeding, after the fascicle had entered a capillary, was always signalized by a sudden change in the character of the blood-flow in the vessel. It was always enormously accelerated, and on some occasions it developed an irregular character, sometimes accelerated, sometimes retarded, resulting in a characteristic 'flickering' appearance in the vessel. So vigorous was the suction exerted that when the fascicle was in a small capillary no blood succeeded in passing its tip, the whole flow being taken up through the orifice of the labrum; this acceleration of the blood-flow was so marked that, if the fascicle was not seen immediately, its location was always indicated by the altered flow of the corpuscles.

So far we have made no reference to the injection of salivary secretion by the mosquito, a point obviously of great importance. Previous literature referring

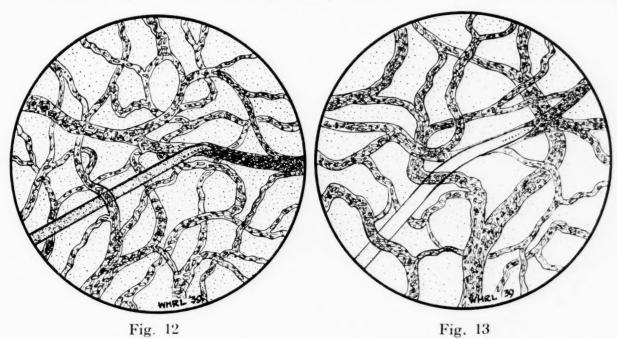


Fig. 12. Capillary feeding in progress. The tip of the fascicle lies in the capillary lumen and is slightly bent. The blood in the labrum is represented by stippling only, as its progress was usually so rapid that individual cells could not be distinguished. The increased blood-flow in the capillary is represented by a denser concentration of cells. (Aëdes aegypti.)

(Semi-diagrammatic; × about 70.)

Fig. 13. Another example of capillary feeding in progress. The fascicle has followed for a short distance the lumen of the capillary. Other explanation as for fig. 12.

(Semi-diagrammatic; × about 70.)

to the injection of salivary secretion is necessarily scanty, since the operation has never before been actually witnessed, and consequently most statements on the subject are in the realm of conjecture. We know that in the case of infected salivary secretion from *Glossina* some of the secretion is injected into the tissues and not into the blood-stream, since trypanosomes can be demonstrated at the site of the bite for a considerable period after inoculation. That a somewhat similar state of affairs follows the injection of sporozoites by mosquitoes is suggested by the work of Boyd and Kitchen (1939) already quoted. In the course of the

experiments now being described, we noted that mosquitoes which were observed to feed for a period up to 30 seconds seldom proved on dissection to have taken up blood, although the reaction produced by the bite was evidence of the injection of salivary secretion. Such observations suggest that part at any rate of the salivary secretion is injected into the tissues before the blood-supply is reached. That the preliminary interrupted feed, of less than 30 seconds, on the human skin did not interfere with the subsequent injection of salivary secretion was proved by allowing a mosquito to bite over a series of interrupted feeds each of 10 seconds' duration. Under these conditions it was found that the reaction following the tenth bite was nearly as marked as that following the first. It is likely that salivary secretion is injected at all stages of penetration by the fascicle, but it is obviously difficult to see small quantities of clear fluid injected into transparent tissues. On three occasions, however, the ejection of fluid from the fascicle was seen to take place; it is not certain that this was saliva, though the volume suggested it. In the first instance, the ejection took the form of three separate gushes of fluid into the tissue while the fascicle was probing towards a capillary. On another occasion, the fascicle, having passed right through the web, entered an air-space between the web and the superimposed cover-slip, which were not in contact at that point; a globule of clear fluid was deposited on the cover-slip, and as the fascicle was withdrawn more fluid was squirted into the space. The third instance indicated that the fluid may be injected into a capillary. On this occasion the fascicle lay in a very small capillary, and a volume of clear fluid was seen to appear below the tip of the labrum—it remained visible between the labrum and the wall of the capillary for a few seconds. It would be unwise to draw any definite conclusion from so small a number of successful observations, but we are inclined to think that salivary secretion is injected during the whole act of biting and is deposited in the tissues, in the lumen of the capillaries, and in any extravasation of blood produced by the mosquito.

Yorke and Macfie (1924) record the fact that, in the case of mosquitoes whose salivary glands contained sporozoites, some of the latter were recoverable from the stomach after feeding, the possibility that these were contaminations from oöcysts in the stomach being eliminated. As Yorke and Macfie point out, this is indicative of the injection of sporozoites by the hypopharynx and their subsequent ingestion by the labrum, passing thence to the stomach. Such an observation does not necessarily suggest the injection of salivary secretion into the tissues but rather implies that it must have been injected either directly into a capillary or else into a pool of extravasated blood in the tissues.

It has been shown, also by Yorke and Macfie, that no agglutinins for human erythrocytes are present in the salivary secretion of Aëdes aegypti; we have carried out experiments using frog's blood with similar negative results. Agglutinins are known to be present in the saliva of Anopheles maculipennis, but we failed to induce this species to bite frogs and were therefore unable to make use of this phenomenon as an indication of the ejection of salivary secretion.

2. The Ingestion of Microfilariae

Microfilariae were, as already noted, abundant in the blood-vessels of the web of the frogs used in these experiments, and their movements in the capillaries could be easily observed. We can confirm the statements of Augustine *et al.* (1936) as to the fact that they are able to hold their position in the small capillaries by pressing their convolutions against the vessel walls, although progress against the blood-stream was rarely seen. In view of the fact that they were so easily observed, we hoped that their actual ingestion might have been studied. On account, however, of the remarkable speed with which the blood is taken up by the fascicle from the capillary we were unable to do so, although on two occasions a microfilaria was seen caught up and wriggling just inside the labrum.

We have already in the introduction referred to two problems relating to the taking up of microfilariae by the mosquito, viz.: the apparent power of a mosquito to take up more microfilariae than can be demonstrated in a similar quantity of blood derived from a finger-prick; and the wide variation in the number of microfilariae taken up by individual mosquitoes from the same host. These two problems seem to us to be closely related, and we propose to deal with that of individual variation first, since the results of our experiments in this connection have helped to a clearer understanding of the apparent concentration of micro-

filariae by the feeding mosquito.

(a) Variations in the number of microfilariae taken up by individual mosquitoes feeding on the same host. We are not aware of any experiments proving that there are constantly greater numbers of microfilariae in the fed mosquitoes than in corresponding amounts of blood taken by skin puncture. By far the most adequate figures are those furnished by O'Connor and Beatty (1937), and a study of their experiments shows that, although the average number of microfilariae taken up by mosquitoes is greater than was found present in the peripheral blood, yet the most extreme variations occur in individual insects. It is noteworthy, however, that this is far more marked in the case of Culex fatigans than in the case of Aëdes aegypti. In Tables I and II we quote the figures given by O'Connor and Beatty.

An examination of these figures shows such wide variations as 738 microfilariae in one mosquito and only 9 in another, although both were fed on the same patient at approximately the same time; a similar marked variation is

recorded by Hinman (1933, 1935) and Galliard (1936).

We have observed a wide variation also in the number of microfilariae taken up by mosquitoes fed on the same frog at similar times; but in our experiments we had the great advantage of being able to witness the whole act of feeding, so that we could correlate the number of microfilariae taken up by a mosquito with the observed method of feeding. Among mosquitoes which fed from capillaries alone fairly wide variations were noted, but the most conspicuous variations were seen when counts of microfilariae in the stomach of mosquitoes feeding from capillaries were contrasted with those observed in mosquitoes

which had been seen to feed on blood extravasated into the tissues. These results are shown in Table III. The numbers recorded are approximate estimates of the actual microfilariae present in the stomach; in cases where the mosquito was not fully gorged we estimated roughly the degree

Table 1*

Comparative number of parasites abstracted by finger-prick and C. fatigans at different hours

Hour	No. of microfilariae in I c.mm. blood at time of mosquito collection	No. of mosquitoes examined	No. of parasites in each insect
6-8 p.m.	8	6	4, 14, 17, 15, 2, 1
8-10 p.m.	14	2	372, 21
10-12 p.m.	9	11	36, 106, 17, 12, 10, 62, 69, 42, 42, 183, 45
12-2 a.m.	7	10	26, 459, 59, 109, 9, 148, 738, 81, 259, 24
2-4 a.m.	7	9	151, 36, 516, 34, 110, 59, 15, 23, 29
4-6 a.m.	5	7	52, 10, 55, 54, 27, 34, 40
6-8 a.m.	Less than 1	-4	13, 21, 4, 21

Table II*

Comparative number of parasites abstracted by finger-prick and A. aegypti at different hours

Hour	No. of microfilariae in 1 c.mm. blood at time of mosquito collection	No. of mosquitoes examined	No. of parasites in each insect
6–8 p.m.	8	5	0, 4, 6, 0, 0
8-10 p.m.	14	3	8, 5, 3
0-12 p.m.	9	2	3, 81
2-2 a.m.	7	4	7, 21, 5, 6
2-4 a.m.	7	4	4, 16, 8, 0
4-6 a.m.	5	4	53, 11, 27, 3
6-8 a.m.	Less than	2	1, 0

of engorgement and applied a correction factor, so as to express the result as the number which would have been present in a fully gorged mosquito. It will be noted that the figures given as controls are derived from samples of the blood obtained from a cutaneous vein. This technique was adopted because

^{*}Quoted from O'Connor and Beatty (1937). The original figures given by these authors for finger-prick blood refer to the number of microfilariae in 20 c.mm. For the sake of easy comparison, we have converted the figures to show the approximate number of microfilariae in 1 c.mm.

it was found impossible to obtain an adequate supply of blood undiluted with lymph by puncturing the web. The number of microfilariae per c.mm. of venous blood is directly comparable with the number found in each fully gorged mosquito, since it is usually accepted that each fully fed A. aegypti takes up approximately this volume of blood.

TABLE III

Showing the number of microfilariae found in the stomach of Aëdes aegypti in cases in which the whole previous act of feeding had been watched; compared with the microfilarial concentration in the venous blood of the frogs

Frog	Experi- ment no.	Stomach; approximate degree of distension	No. of microfilariae	Corrected no. of microfilariae	Type of feeding
No. 13	9	3 4	15	20	Pool
Average no, of micro-	11	1	94	94	Capillary
filariae per c.mm.	35	1	13	17	Pool
of venous blood	36	1	207	207	Capillary
178	39	1/5	0	0	Pool
	40	1	178	237	Capillary
No. 7	51	1	circa 1,300	circa 1,300	Capillary
Average no. of micro-	57	3	., 500	670	Capillary
filariae per c.mm.	81	3	., 900	1,200	Capillary
of venous blood 405	85	1	1,500	,. 1,500	Capillary

It can be seen from Table III that, as is recorded by other observers, the number of microfilariae taken up by individual mosquitoes varied greatly. That this variation was not due to any larval periodicity was proved by the counts of the microfilariae in the venous blood remaining more or less constant at various hours of the day and night; Yorke and Blacklock (1917) have shown that in the case of the periodic larva of *Wuchereria bancrofti* the curve of the venous periodicity is synchronous with that of the cutaneous blood, and, further, that the concentration of larvae in the cutaneous blood is greater than that in the venous blood. Our figures for capillary blood feeding are also, with one exception, higher than the venous blood values.

Although the number of cases in which we were able to convince ourselves that pool feeding alone took place is small (three), yet the difference in microfilarial concentration is so marked that the figures indicate, as already stated, that, when the blood is taken up from a pool extravasated into the tissues, the number of microfilariae is less than when direct feeding on the capillary takes place. We believe this difference to be due to the fact that erythrocytes escape from a torn capillary more readily than do microfilariae.

This hypothesis would explain some of the low counts recorded by ourselves and other observers as due to the mosquitoes having obtained their blood by pool feeding. It does not, however, account for the marked variations also met with when microscopical examination had shown that only capillary feeding took place, though these variations, in the small number of observations noted, do not seem to be so wide. We believe that this variation in the concentration of microfilariae taken up during capillary feeding may be very simply explained. A series of direct examinations of the webs of infected frogs under the microscope showed that the number of larvae in closely adjacent capillaries varied in a most remarkable way. Often microfilariae were congregated in large numbers over a short length of a capillary, so that the latter was occupied by a writhing mass of larvae with very few blood-cells, at a time when in neighbouring capillaries microfilariae were very rarely seen. It would seem, therefore, that under these circumstances the number of microfilariae ingested would depend on the particular capillary from which the mosquito chanced to feed.

O'Connor and Beatty (1937) suspected that such variations might be due to the different depths to which mosquitoes penetrate in feeding, and perhaps to the size of the vessels from which they draw blood, considering it likely that the microfilarial concentration would be higher in narrow, tortuous, than in

wide, straight, vessels.

Although we have been unable directly to prove this latter suggestion by observation, it seems highly probable that it is correct, for we have noted the fact that in small capillaries the microfilariae progress more slowly than the corpuscles, since their writhing movements constantly bring their coils in contact with the walls of the vessel. Under these circumstances they often hold their positions in the capillaries, sometimes allowing the corpuscles to pass and sometimes causing stasis. The fact that they can, to some extent, resist being swept away implies that they progress more slowly in the smaller capillaries than the corpuscles, and therefore that their concentration relative to the corpuscles will be increased in those sites. Examination of the microfilariae in large capillaries, however, can only be made when the circulation is completely stopped, which necessity prevented our proving the hypothesis by direct observation.

(b) The apparent power of a mosquito to take up more microfilariae than can be demonstrated in a similar quantity of blood derived from a finger-prick. Manson's original hypothesis (1883) to explain high microfilarial counts in mosquitoes, which has been supported by O'Connor and Beatty (1937), was that microfilariae may become entangled round the proboscis as they do round strands of cotton. No such phenomenon was observed by us during the course of our experiments, and apparently the microfilariae were freely sucked up during the extremely rapid rush of blood up the labrum.

Another hypothesis put forward by certain writers, notably Harley (1932), is that the injection of salivary secretion by the mosquito may attract the microfilariae in the area to the fascicle. Anyone who has watched blood being drawn

up from a capillary by a mosquito cannot fail to be impressed by the extraordinary acceleration of the blood-flow which is produced. It seems impossible that any chemotropism exerted by salivary secretion would be effective under such conditions. It is true that Strong's results (1934) suggest a regular concentration of microfilariae in an insect's stomach, such as would be caused by a chemotropism, but his results only apply to *Onchocerca volvulus* larvae taken up by *Simulium*—the habits of these microfilariae are widely different from those of the blood-inhabiting microfilariae, as is also the biting mechanism of the fly from that of the mosquito.

Our own observations do not support the theory that the greater concentration of microfilariae in the mosquito is due to any mechanical power of the proboscis to pick up microfilariae, or the belief that a chemotropism attracts the microfilariae to the site of the bite. As a result of our experiments, we believe that the blood taken up by a mosquito during capillary feeding is a true indication of the microfilarial concentration in that particular vessel, and as, in our experience, mosquitoes feed on the smaller capillaries, which tend to have the highest concentration of microfilariae, therefore the concentration of microfilariae in the stomach is greater than that in blood obtained from a finger-prick, which sample is derived from capillaries and venules of all sizes. Further, the pricking of the finger results in a laceration of the capillaries, from which laceration the microfilariae escape less readily than the blood-cells.

IV. SUMMARY

1. A technique is described, using the web of a frog, by which observations may be made on the mouth-parts of a mosquito while penetrating living tissue, on the ejection of fluid (presumably salivary secretion) by them, and on their taking up of blood.

2. The mechanism of penetration is found to differ markedly from previous observers' conceptions. The fascicle is found to be actively flexible at its tip, which can be curved to an extent allowing of penetration in almost any direction. The remainder of the fascicle appears to be passively flexible and able to accommodate itself to the curves previously pursued by the cutting tip.

3. Two distinct methods are recorded by which a mosquito takes up blood: (1) directly from a capillary, (2) from an extravasation of blood derived from a previously lacerated capillary.

4. It is shown that fluid, presumably salivary secretion, is injected into the tissues at various stages of penetration. It is probable that the saliva gains access to the tissues, to any haemorrhage that may have been produced by the mosquito, and directly to the capillary circulation.

5. The observations of other authors regarding the independent movement of microfilariae against the blood-stream are, in part, confirmed. On the other hand, we fail to find any evidence supporting the suggestion that microfilariae are capable of purposive movement towards the site of feeding.

6. Attention is drawn to the extreme variation which may occur in the numbers of microfilariae taken up by individual mosquitoes fed on the same host.

It is shown that this may be correlated to some extent with different types of feeding, feeding from blood extravasated into the tissues resulting in a much lower concentration of microfilariae than when blood is taken up directly from a capillary. This is probably due to the fact that microfilariae escape less readily from a torn capillary than do blood-cells. Another cause of the variation is that the concentration of microfilariae varies considerably in different capillaries.

7. It is a well-recognized fact that, on the average, mosquitoes take up a relatively greater concentration of microfilariae than is to be found in fingerprick blood Evidence is produced which suggests that this phenomenon is due neither to a chemotropism nor to any mechanical power of the proboscis to entangle microfilariae. It appears probable that the microfilarial concentration observed in the stomach of a freshly gorged mosquito is a true indication of their concentration in the particular capillary from which it has fed, and we believe that high counts are to be explained by microfilariae stagnating in small capillaries, our experiments showing that it is from such small capillaries that a mosquito most often feeds. The discrepancy between the concentrations of microfilariae in the mosquito's stomach and in finger-prick blood may be further explained by the suggestion, already referred to, that corpuscles escape more readily from lacerated capillaries than do microfilariae.

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AN INSECTARIUM WITH CONSTANT TEMPERATURE AND HUMIDITY CONTROL; TOGETHER WITH A DESCRIPTION OF A SIMPLIFIED TECHNIQUE FOR THE REARING OF ANOPHELES MACULIPENNIS VAR.

ATROPARVUS

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INTRODUCTION

The insectarium which we are about to describe was designed after we had visited a number of laboratories, and we are indebted to the following persons not only for their advice, but, in certain instances, for supplying us with various insect strains: Professor P. A. Buxton and Dr. C. Johnson, of the London School of Hygiene and Tropical Medicine; Dr. B. Jobling, of the Wellcome Field Research Laboratory, Esher, Surrey; Mr. J. B. Marshall, of the British Mosquito Control Institute, Hayling Island; Professor K. Rose, of the Robert Koch Institute, Berlin; Lt.-Col. J. A. Sinton and Mr. P. G. Shute, of the Ministry of Health Malaria Laboratory, Epsom; Dr. P. Tate and Dr. A. Bishop, of the Molteno Institute of Biology and Parasitology, University of Cambridge. We have also to thank Mr. P. J. Robinson, Liverpool City Electrical Engineer, for much helpful advice.

DESCRIPTION

The insectarium is built on the first floor of the Liverpool School of Tropical Medicine. The general design is shown in figs. 1 and 2. It consists of a room measuring 12 ft. \times 10 ft. 2 in., with the ceiling at the highest point 11 ft. 6 in. above floor-level. This small room lies between two larger rooms and is separated from them by walls of lath and plaster; being hollow, these walls act as efficient non-conductors of heat. The outside wall is of brick and concrete, 1 ft. 9 in. thick.

The floor is constructed of the same red asphalt material as exists in the rest of the building. In some English insectariums the floors are kept flooded, or else large pans of water under the heaters are provided, in order to maintain

a high humidity, but, as will be seen later, in the insectarium now being described the provision of automatic humidifiers renders this unnecessary.

The ceiling in the original room is 11 ft. 6 in. high, but a false ceiling has been introduced which covers one half of the insectarium to a height of 8 ft. 8 in. and then slopes up sharply to join the outer wall, leaving an air-space between

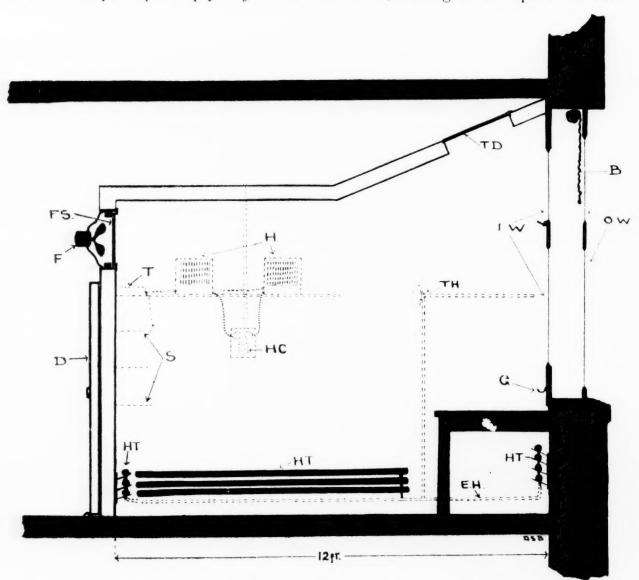


Fig. 1. An elevation to show the construction of the insectarium and the arrangement of the heaters, thermostat and humidity control.

B.—aluminium blinds; D.—door; E.H.—wiring for heaters; F.—fan; F.S.—shutter over fan aperture; G.—gutter for drainage of water of condensation from window; H.—humidifier; H.C.—humidity control; HT.—heater; I.W.—inner window; O.W.—outer window; S.—shelving; T.—tank; T.D.—trap-door; TH.—thermostat.

the old and new ceilings. This arrangement has the advantage of reducing the volume of air to be maintained at a constant temperature and humidity while admitting the maximum amount of light. The space above the false ceiling acts as a non-conductor of heat and also limits the escape of warm damp air which might cause rotting of the building.

The treatment of the surface of the walls and ceiling is of considerable importance. Under the required conditions of temperature and humidity, paper and paint were considered to be unsatisfactory, and we finally adopted the method used at the Ministry of Health Malaria Laboratory, Epsom. This consists of roughing the plaster walls to provide a good grip for a surface-dressing of a thick paste of whiting and size-water to which has previously been added a small quantity of carbolic, in order to reduce fungus growth. We found it advisable to coat the whitewash when dry with a waterproof varnish.

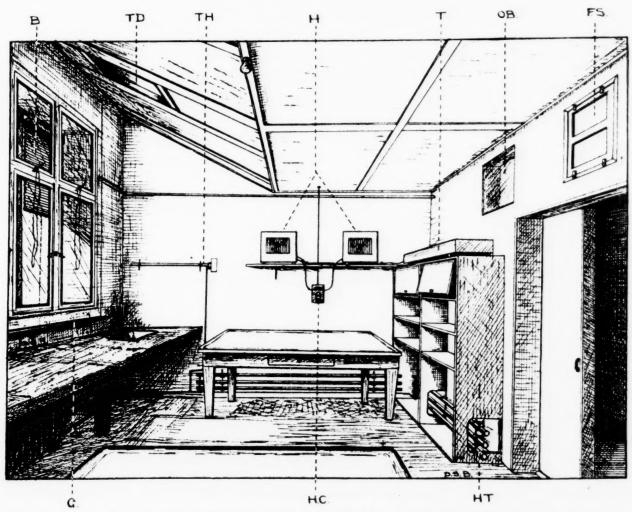


Fig. 2. The interior of the insectarium, showing the arrangement of the equipment. OB.—observation window. Other lettering as in fig. 1.

A sliding door is provided, which gives access to a narrow corridor running outside the insectarium, thus reducing air-disturbances when entering or leaving the room.

Daylight is admitted through one large window, measuring 7 ft. square, at the south end of the room. The window is double, with a space of 12 in. between the outer and inner panes, and sections of both outer and inner windows can be opened. The object of a double window is to conserve heat, since even

with a double window of this type it is calculated that one quarter of the total heat lost from the room is by radiation through the window. During winter, condensation on the inner surface of the glass is sufficiently great to necessitate the provision of a drain to protect the bench below. The advisability of fitting vita-glass was considered, but neither the necessity for this nor for employing an ultra-violet ray lamp has as yet arisen. A small double observation window on the passage wall is also provided.

Heating is supplied by non-radiant heat, the resistances being enclosed in tubular heaters which are carried in brackets away from the wall and suspended sufficiently high above the floor-level as not to interfere with the cleaning of the room. The heaters are connected to two thermostats, of the usual metal expansion type, placed about 6 ft. above floor-level and situated one on each side wall, as indicated in the figures. The thermostats are set to keep the temperature between 23° and 25° C., one thermostat controlling three groups of heaters, the other the remaining two. We were warned, as a result of visiting other institutions, to keep all wiring outside the walls and to see that it was enclosed in metal tubing. Failure to observe these precautions under the required conditions of high humidity is likely to be followed by short circuits. Contact between the heating installation in the room and the main supply is controlled by a switch located in the corridor outside the insectarium.

For general purposes we wished to maintain a relative humidity of between 75 and 85 per cent. at the selected temperature of between 23° and 25° C. Although the temperature remains the same, the humidity is bound, of course, to vary in the different jars and gauze cages. Experience showed that in the absence of any humidifying apparatus, but with large areas of water exposed, the room if undisturbed settled to a relative humidity of nearly 70 per cent. We have already stated that in some laboratories flooding of the floors is used as a means of raising the humidity. Other methods commonly employed include the use of water-sprays or allowing the escape of steam; all these methods have obvious disadvantages and are difficult to control automatically. We have obviated some, at any rate, of these disadvantages by installing two 'Air Conditioning Cabinets' made by the A.E.G. Electrical Co. Ltd. Each cabinet contains a number of vertical sheets of absorbent paper which are suspended from a frame so that their lower ends are immersed in a trough of water forming the base of the cabinet; by means of an electric fan behind the sheets a current of air can be directed across the wet sheets into the room. Each cabinet holds nearly half a gallon of water, but this was not sufficient for our purpose, and we therefore connected both humidifiers with a large tank holding some 5 gallons. It was found that when the insectarium was kept at 24° C. the two humidifiers consumed, in winter, approximately 2½-4 litres of water during the 24 hours, when maintaining an average relative humidity of 79 per cent. The humidifiers as supplied have no automatic control and we therefore installed a humidity control manufactured by Messrs. Honeywell-Brown, Ltd. The hygroscopic element,

consisting of human hair operating a mercury switch connected with the motors driving the fans, is situated just above bench-level, while the humidifiers are on a shelf some 6 ft. above floor-level.

Considerable difficulty was experienced in arranging for suitable lighting. With so small a room and so relatively large a window, exposed as it is to the south, there is considerable danger of sudden excessive rises of temperature in summer, experience proving that even in early summer moderate direct sunshine raises the temperature to nearly 30° C. (see Chart 2). In order to obviate this danger, blinds were introduced between the sheets of glass in the double window; outside blinds would probably have been more satisfactory, but this proved impracticable. Our first intention was to have aluminium strips arranged in the form of a venetian blind, but the cost of these was prohibitive, the lowest estimate placed at our disposal being some £17. Finally we installed, at the cost of a few shillings, roller blinds consisting of corrugated cardboard faced on the outer surface with aluminium foil. These are inserted in the space between the two sets of panes and are wound on rollers operated from inside the room. As an additional precaution against sudden rises of temperature in summer, and also to ventilate the room after the floor has been washed, etc., an exhaustfan drawing air from the corridor into the room is provided. When this is in use a trap-door, situated on the sloping part of the roof, is raised by means of pulleys operated from the corridor. A current of air is forced, in this way, to pass through the room, and causes a rapid fall in the temperature and humidity. When the fan is not in use, the fan aperture is closed by a shutter to prevent the escape of warm damp air.

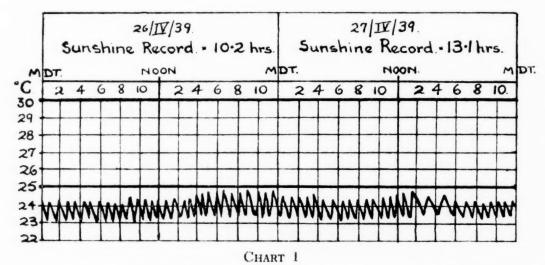
The cost of constructing the insectarium and installing the necessary fixtures was approximately £123, the structural alterations costing some £65 and the electrical fittings and equipment some £58. During a year the total consumption of electricity by the heaters and humidifying fans was just under 5,000 units.

RESULTS

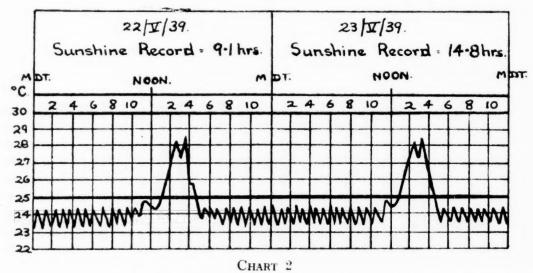
The insectarium has now been running for some 18 months and has given very satisfactory results, the following routine strains having been maintained in it during this time: Anopheles maculipennis var. atroparvus, Aëdes aegypti, Culex molestus, Xenopsylla cheopis, Cimex lectularius, C. hemiptera, Rhodnius prolixus and Ornithodorus moubata.

During eleven weeks, from April to July, 1939, we kept drum records of the temperature and humidity. As regards temperature, the records show that it is impossible to maintain a constant temperature without the use of blinds. When the blinds were in position the temperature fluctuated within a range of only 2° C. (see Chart 1). On the other hand, Chart 2 shows that, when the blinds were not used, sudden rises of temperature extending to as much as $4\frac{1}{2}^{\circ}$ C. occurred on sunny days, even in early summer,

As regards the control of the relative humidity in the insectarium, it was found possible to maintain constantly a relative humidity of between 75 and 85 per cent., so long as reasonable precautions were taken to see that the door was not left open unnecessarily. Chart 3, section A, illustrates the relative humidity maintained under normal working conditions. The humidifiers, in addition to maintaining the required humidity under normal conditions,



Showing the uniform temperature maintained in the insectarium in early summer when the aluminium blinds are in use.



Showing marked variations in temperature which occur in early summer when blinds are not used.

are also able rapidly to build up the humidity when, for any reason, such as the accidental leaving open of the door, a sudden fall in the humidity has been allowed to occur.

When the humidifying fans are disconnected and the door of the insectarium is allowed to remain open, the relative humidity falls rapidly to about the same as

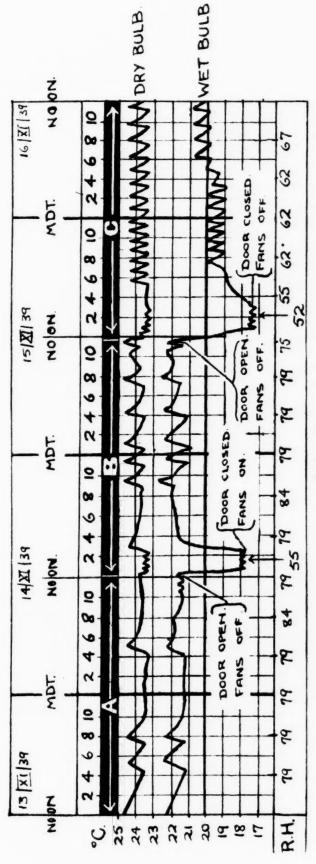


CHART 3

switching off of the humidifiers. It also shows the rapid building up of the relative humidity to the A continuous record over three days showing the relative humidity normally maintained in the insectarium and the value of the humidifiers in producing and maintaining the desired humidity. Section B shows a sudden drop in the relative humidity following the opening of the door and the illustrates how, in the absence of humidifiers, a sudden drop in humidity, following the opening Section A shows the uniform relative humidity maintained under normal working conditions. Section C of the door, is not succeeded by a rapid rise in the relative humidity when the door is again closed. It also shows that in the absence of humidifiers the relative humidity remains below 70 per cent. required 80 per cent. when the door is closed and the humidifiers are switched on.

The tracing was made with self-recording wet-and-dry-bulb thermometers of the mercuryin-steel type manufactured by Messrs. Negretti and Zambra. The relatively smaller frequency the tracings in Charts 1 and 2, are due to the presence of an unprotected central-heating pipe in the The increased frequency following the insulation of the pipe towards the end of the of the temperature changes in sections A and B as compared with the remainder of the chart, and with experiment is clearly shown. insectarium.

that in the rest of the building (50–60 per cent.), at which level it remains fairly constant. If the doors are then shut and the humidifiers switched on, a rapid rise in the relative humidity takes place. Under these conditions about $1\frac{1}{2}$ hours are required to reach a relative humidity of 79 per cent., at which it is maintained unless the door is again left open. This sequence of events is shown in section B of Chart 3.

If, however, a similar experiment is carried out in the absence of humidifiers, a very different result is obtained. Instead of $1\frac{1}{2}$ hours being required to build up a constant relative humidity after the door has been closed, some 15 hours are necessary to reach a figure of 67 per cent., no further rise being possible so long as the fans are not in action. This slow rise in the relative humidity is shown in Chart 3, section C.

A SIMPLIFIED TECHNIQUE FOR THE REARING OF ANOPHELES MACULIPENNIS VAR. ATROPARVUS

The strain was supplied to us in July, 1938, by the courtesy of Mr. P. G. Shute, from the Malaria Laboratory of the Ministry of Health, Epsom, and has been maintained successfully from that date up to the present time in the insectarium described above.*

The strain was at first reared according to the technique described by Shute (1936); this method yielded uniformly good results, but had the disadvantage of entailing a considerable expenditure of time in the collection of pupae from amongst the lining sods of grass which fringe the edges of the artificial pool. Another disadvantage is that the circular earthenware pans, over 18 in in diameter, are uneconomical as regards space, their size and weight rendering them rather unsuitable for a small laboratory. Finally, the artificial pools have to be re-made every few weeks.

In August, 1938, one of us (R.M.G.) was instructed by Dr. M. Bates, of the Rockefeller Institute, regarding a technique successfully used by him at the Institute's laboratory in Albania. Dr. Bates's technique as described to us is briefly as follows.

The soil is collected in a bucket and brought to the laboratory; it should be top soil, with or without adherent grass. Ordinary field soil is quite satisfactory; so-called sandy loam is best, while clay should on no account be used. It is also necessary to see that no fertilizer has previously been added to the soil, and that it has not been chemically treated in any way. Having been brought to the laboratory the soil is mixed in a bucket with water—preferably rainwater. The mud and water are then puddled with the hands and strained through mosquito-gauze to remove sticks and stones, the larger particles of earth, and any insect enemies. The mixture is then allowed to stand in the

^{*}After the outbreak of war a temporary reduction in staff caused the stock to become depleted; the strain has now been renewed from the original source.

bucket overnight, and on the following day some of the supernatant fluid is suctioned off, leaving a proportion of two parts of mud to one part of water. This represents the stock food-supply for the larvae, and should remain in a suitable condition for at least a month, probably for two or three months. required, the mud and water are vigorously mixed together, and the desired amount is diluted with rather more than an equal quantity of rain-water. a new culture is being started, 200-400 eggs are hatched in a small white enamel bowl, the eggs being confined in the usual float. For the newly hatched first stage larvae the optimum food-supply is 1 c.cm. of the prepared mud and water to each larva; to this must now be added breadcrumb. breadcrumb is dried in the oven, ground very finely, passed through a closemesh sieve and placed in a container covered with a perforated top which is lined with voile. By using this sprinkler, a very fine layer of breadcrumb can be spread on the surface of the water, and this dusting of the surface should be repeated every day throughout larval life. When this method is used at a temperature of about 22-24° C., larvae should be well into the second instar in about 4-5 days. At the end of this period the mud is stirred up and, together with the larvae, is poured into a larger enamel dish. After this has been done, the bulk of the fluid is increased by adding fresh mud and water, to such an amount that each larva will have a double supply, i.e., 2 c.cm. per larva. The larvae are now left in this dish, to which the breadcrumb is added daily, until they reach the fourth instar, which should occur 4 or 5 days later. When this has occurred they are transferred into another dish large enough to contain an average volume of 5 c.cm. of mud and water for each larva.

It will be seen from this description of Bates's technique that an increased amount of mud and water is supplied at each stage of the larval growth. It is important to note that Bates considers the increase of surface area per larva of more importance than any increase in the depth of the fluid. To conform with these requirements we use shallow enamel baking-dishes of about the same depth but of successively greater area, the depth of mud and water recommended by Bates being about 2 in.; in our laboratory we find 1 in. sufficient.

This technique gave us equally good results, as regards the rearing of large healthy adults from ova, as that recommended by Shute, and had the advantage of economizing both time in the collecting of the pupae and space in the room.

The only drawback which we observed in Bates's method was that, when a suitable source of earth was discovered, cultures made up from it by Bates's technique did not keep indefinitely, and it was then necessary to re-visit the source of the soil in order to obtain a further supply. In our case this necessitated a considerable journey, and it was found that the nature of the soil varied considerably at different times of the year. It occurred to us that it might be possible to dry large quantities of earth obtained from a suitable site and to preserve it in powdered form until it was required. This was found to be quite practicable, and our final technique is now as follows.

Earth is obtained in the manner described by Bates; in practice we obtain our supply from a field about 10 miles from Liverpool, the grass-covered sods being dug up from the top of a bank bordering a small stream in which A. bifurcatus breeds.

The earth is brought back to the laboratory, and the sods, with grass attached, are broken up by hand, spread over newspapers and allowed to dry in a warm room; the earth is then further thinned by being passed through different wire meshes. Finally, it is stored in 5 lb. fruit-jars which are sterilized in a hot-air oven at 160° C. for 20 minutes on two successive days. When required for use, 9 parts of the prepared earth are mixed with 50 parts of rain-water, although, in our laboratory, Liverpool tap-water has proved equally satisfactory. The mixture is then allowed to settle and the particles which float to the surface are skimmed off; it is necessary to get rid of these because, when present in quantity, they appear to inhibit proper development of the larvae. The resultant mixture forms the first breeding-dish for the growing larvae. At intervals, as recommended by Bates, additional mixture is prepared in a suitable dish, and the larvae, together with the original medium, are transferred to it.

Using this technique we have had similar excellent results to those obtained with the original Bates technique, and have had no difficulty in rearing stocks of large healthy adults.

REFERENCE

SHUTE, P. G. (1936). A simple method of rearing and maintaining Anopheles maculipennis throughout the year in the laboratory. H. Trop. Med. & Hyg., 39, 233.

STUDIES IN CHEMOTHERAPY*

XXI.—THE TRYPANOCIDAL ACTION OF CERTAIN AROMATIC DIAMIDINES

BY

E. M. LOURIE

AND

WARRINGTON YORKE

(Received for publication July 25th, 1939)

In previous papers (King, Lourie and Yorke, 1937, 1938) the results are recorded of the examination for trypanocidal activity of a considerable number of guanidines, isothioureas, amidines and amines with alkyl and alkylene chains. It was found that some of these compounds exhibited a powerful trypanocidal action *in vitro* and that with certain of them, notably n. undecane-1: 11-diamidine, it is possible to produce permanent cures in approximately 100 per cent. of mice and rabbits infected with our laboratory strain of *T. rhodesiense*.

The structural feature common to all these compounds is the possession of a central inert carbon chain with terminal polar groups of strongly basic nature; and as it seemed possible that this carbon chain merely served as a carrier of the active groupings, and might be replaced by an inert aromatic structure of approximately the same molecular weight, a number of aromatic amidine and guanidine compounds were prepared. Several of these compounds were found to exhibit trypanocidal action.

Immediately following the publication of the paper by King, Lourie and Yorke (1937) referred to above, Dr. A. J. Ewins, of Messrs. May and Baker, Ltd., took up the investigation of a series of aromatic compounds containing the amidine group. The first member of the group so examined, 4:4'-diamidino diphenyl methane, exhibited appreciable trypanocidal activity experimentally in vivo, and thus showed that an extension of the investigation on these lines was desirable. A large number of derivatives were therefore prepared by the members of the research staff working under his direction, in which a number of factors, such as variation of the length of the alkane chain linking the amino-phenyl residues, replacement of the methylene groups by various divalent

^{*} This work was assisted by grants from the Medical Research Council and from Messrs. May and Baker, Ltd.

groups and atoms, substitution, etc., were examined. The following list of compounds indicates the general lines on which the investigation was developed. A full account of this work will be published shortly in another place. In this list R represents $\stackrel{NH}{NH_2}C$ and the figures represent the position of the amidine group in the benzene nucleus relative to the point of attachment of the linking chain.

A. -COMPOUNDS LINKED BY A STRAIGHT -C- LINKAGE

- 4: 4'-R-CH₂-R
 4: 4'-diamidino diphenyl methane
 4: 4'-R-CH₂CH₂-R
 4: 4'-diamidino diphenyl ethane
 4: 4'-R-CH₂CH₂CH₂-R
 4: 4'-diamidino diphenyl propane
 4: 4'-R-CH: CH-R
 3: 4'-diamidino stilbene
 4: 4'-R-CO-R
 4: 4'-diamidino stilbene
 4: 4'-diamidino benzophenone
- 4:4'-R-CH:CHCO-R
 4:4'-diamidino benzalacetophenone
- 4:4'-R-CHOH-R 4:4'-diamidino benzhydrol

B.—Compounds Linked by an -O- Linkage

ne:
1.

C .- COMPOUNDS LINKED BY AN -N- LINKAGE

4:4'-R-CH ₂ NHCH ₂ -R	4:4'-diamidino dibenzyl amine
4: 4'-R-CH ₂ NH-R	4:4'-diamidino benzyl aniline
4:4'-R-N:N-R	4:4'-diamidino azo benzene
4:4'-R-NHCONH-R	4:4'-diamidino diphenyl urea
4:4'-R-O(CH ₂) ₂ NH-R	4: 4'-diamidino β -phenoxy ethyl aniline

D. - COMPOUNDS LINKED BY AN -S- LINKAGE

4:4'-R-CH ₂ SCH ₂ -R	4:4'-diamidino dibenzyl sulphide
4:4'-R-SO ₂ -R	4: 4'-diamidino diphenyl sulphone
4: 4'-R-SO ₂ NH-R	4:4'-diamidino benzene sulphonanilide
4:4'-R-SS-R	4: 4'-diamidino diphenyl disulphide

E. - MISCELLANEOUS ACTIVE AMIDINES

$$CH_{2}$$
 CH_{2}
 CH_{2}
 CH_{2}
 CH_{2}
 CH_{2}
 CH_{3}
 CH_{4}
 CH_{4}
 CH_{5}
 CH_{5}
 CH_{5}
 CH_{5}

F.-MISCELLANEOUS INACTIVE AMIDINES

Dr. Ewins' preliminary tests on infected mice showed that most of the compounds in Groups A, B, C and E exhibited trypanocidal activity, but those in Groups D and F were either without action or active only to a slight degree.

The present communication records the results of more extensive examination of the compounds which possess the highest degree of trypanocidal activity. The results obtained by intraperitoneal administration of these various diamidines to mice infected with our laboratory strain of *T. rhodesiense* are summarized in Table 1.

Showing the results of treating mice infect

Com R represents NH	npound				Mgm	. per :	20 gm
R represents NH		0.005	0.00625	0.01	0.0125	()	.025
4:4'-R-CH ₂ -R	4: 4'-diamidino diphenyl methane						
4: 4'-R-CH ₂ CH ₂ -R	4: 4'-diamidino dipheny! ethane			5 5N		.,	δR
4:4'-R-CH ₂ CH ₃ CH ₅ -R	4 : 4'-diamidino diphenyl propane						
4: 4'-R-CH: CH-R	4 : 4'-diamidino stilbene	16 7N 9R	15 5N 10R	15 13R 2C	16 10R 6C	21	6R 15C
4:4'-R-CH:CHCO-R	4 : 4'-diamidino benzal- acetophenone						
3 : 4'-R-CH : CH-R	3 : 4'-diamidino stilbene			2 2N		4	IN 3R
4 : 4'-R-O-R	4 : 4'-diamidino diphenyl ether			7 7N		13	5N 8R
4: 4'-R-CH ₂ O-R	4: 4'-diamidino phenyl benzyl ether	6 6N		10 8N 2R	10 2N 8R	21	20R
3 : 4'-R-CH ₂ O-R	3 : 4'-diamidino phenyl benzyl ether			2 2N	4 4R	4	‡R
4:4'-R-CH ₂ CH ₂ O-R	4: 4'-diamidino β-phenyl ethyl phenyl ether	B. Makers region in recovery against legit filtre title a		e , i galagang ur 1994-1998 h-saighteánn t-reanaigh neag	er Aggingage for Bermille berk segundarungen verbere		
4:4'-R-CH ₂ OCH ₂ -R	4 : 4'-diamidino dibenzyl ether	,		5 5N		,)	:R
4:4'-R-OCH ₂ OCH ₂ O-R	4:4'-diamidino diphenoxy dimethyl ether						
4:4'-R-CH ₂ OC ₆ H ₄ OCH ₂ -R	1 : 4-di-(4'-amidino benzyloxy)-benzene	A North of the Control of the Contro					
1: +'-R-OCH ₂ C ₆ H ₄ CH ₂ O-R	ω: ω'-di-(p-amidino phen- oxy)-xylene						

These diamidines were in all cases used in the form of their dihydrochlorides, to which the figures in the tables actual refer.

N = Blood did not become negative. R = Blood became negative, but a relapse occurred. C = Permanent cure.

ected of T. rhodesiense with various aromatic diamidines

nio	use give	en int	raperito	oneall	ì.							M.E.D.	M.C.D.	M.T.D.	Remarks
	0.05		0.1	(0.25		0.5	,	1.0		2.0				
16	16N	3	3R	37	27R 10C	37	16R 21C	37	6R 25C 6P	36	22P 2R 12C	0.1	1.0	1.0	
5	4R 1C	5	2R 3C	5	2R 3C	5	5C	9	7C 2P	4	4P	0.025	0.25 0.5	1.0	
												0·05 0·05	0·2 0·5	0·4 1·6	Intraven. Subcutan. Ewins
26	3R 23C	14	IR 13C	4	4C	5	5C	11	10C 1P	6	5P 1C	0.00625	0.025/0.05	1.0	
					the transfer of the second	The state of the s						Nil Nil	Nil Nil	1·0 2·0	Intraven. Subcutan. Ewins
1	4R	4	1R 3C	3	1R 2C	4	4C	4	3P 1C	2	2P	0.025	0.25/0.5	0.5	
11	11R	13	7R 6C	14	2R 12C	14	2R 12C	19	7P 12C	11	ПР	0.025/0.05	0.25	0.5/1.0	
20	10 R 10 C	20	4R 16C	5	5C	5	5C	11	2P 9C	11	2P 9C	0.0125	0.1	1.0/2.0	
+	3R 1C	4	3R 1C	4	1R 3C	4	4C	4	4C	2	2P	0.0125	0.25	1.0	
												0·05 0·05	0·3 0·8	0·3 1·2	Intraven. Subcutan. Ewins
ā	IN 4R	5	5R	5	2R 3C	5	5C	9	9C	4	4P	0.025	0.5	1.0	
												Nil Nil	Nil Nil	0·4 1·6	Intraven. Subcutan Ewins
												0.8	Nil 1·2	0·2 2·4	Intraven. Subcutan. Ewins
												0.1	Nil 1·2	0·05 2·4	Intraven. Subcutan. Ewins

The numbers on the left-hand side of columns 3-13 ('Mgm. per 20 gm. mouse given intraperitoneally') show the number animals treated; those on the right-hand side show the result of treatment.

M.E.D. = Minimum effective dose. M.C.D. = Minimum curative dose. M.T.D. = Maximum tolerated dose.

Showing the results of treating mice infecte

	\	mpound								Mgm	. per	20 gm
R represer	ars	$_{\mathrm{H}_{2}}$ $^{\circ}$ C $^{\circ}$	()	-005	0.0	00625		0.01	()	0125	0	0.025
\$: 4'-R-OCH ₂ O-R		4: 4'-diamidino diphenoxy methane			age allowed and demonstrate	grand standards precessaries	2	2N		eta e e e e e e e e e e e e e e e e e e	6	2N 4R
4:4'-R-O(CH ₂) ₂ O-R		4 : 4'-diamidino diphenoxy ethane	.5	5N			9	9N	5	4N IR	7	IN 6R
4:4'-R-O(CH ₂) ₃ O-R		4 : 4'-diamidino diphenoxy propane	.5	5N	- Participation		9	6N 3R	20	6N 14R	20	13R 7C
3 : 3'-R-O(CH ₂) ₃ O-R		3: 3'-diamidino diphenoxy propane .					2	2N	-		1	IN 3R
4:4'-R-O(CH ₂) ₅ O-R	b 0 0	4:4'-diamidino diphenoxy pentane	15	IIN 4R	15	11N 4R	16	2N 14R	16	2N 13R 1C	20	10R 10C
3:3'-R-O(CH ₂) ₈ O-R		3 : 3'-diamidino diphenoxy pentane							a landana de la la landana de			
4:4'-R-O(CH ₂) ₁₀ O-R		4: 4'-diamidino diphenoxy decane										
4:4'-R-O(CH ₂) ₂ NH-R	0 0 0	4: 4'-diamidino β -phenoxy ethyl aniline	•)	2N			4	3N IR	10	4N 6R	7	IN 6R
4 : 4'-R-NHCONH-R		4 : 4'-diamidino diphenyl urea	I PRINT AT HERE SINCE								Section of	

These diamidines were in all cases used in the form of their dihydrochlorides, to which the figures in the tables actually reference.

N Blood did not become negative. R Blood became negative, but a relapse occurred. C Permanent cure.

It is seen that many of the compounds exhibit considerable trypanocidal activity, and that some of them do so in a quite remarkable degree. The most active is apparently 4:4'-diamidino stilbene:

This compound is tolerated by mice when administered intraperitoneally in doses up to 1 mgm. per 20 gm. mouse; such minute doses as 0.005 and

(Continued)

th T. rhodesiense with various aromatic diamidines

	ouse give	n intr	aperito	neally	·							M.E.D.	M.C.D.	M.T.D.	Remarks
	0.05		0.1	0	.25		0.5		1.0		2.0				
	IN 5R IC	5	2R 3C	5	2R 3C	11	9C 2P	11	3C 8P	7	7P	0.025/0.05	0.25/0.5	0.5	
	3 11R 2C	15	2R 13C	5	5C	9	9C	16	14C 2P	11	HP	0.025	0.1	1.0	
	8 8R 18C	28	5R 23C	7	7C	9	9C	16	8C 8P	11	11P	0.0125	0.05/0.1	0.5/1.0	
	3R 1C	4	4R	4	4R	4	4C	4	4C	2	2P	0.025	0.5	1.0	
	8R 12C	20	4R 16C	6	6C	6	6C	10	7C 3P	9	9P	0.01	0.05/0.1	1.0	
-		-	and the same of the same of				nesta. Allegar ya V sanga	-				0·1 0·1	Nil 0·4	0·5 1·6	Intraven. Subcutan. Ewins
												0·05 0·05	Nil 0·5	0·8 3·0	Intraven. Subcutan. Ewins
	3R	10	3R 7C	10	2R 8C	5	5C	8	8C	8	8P	0.025	0.1/0.25	1.0	
												0·1 0·4	0·4 0·8	0·4 1·2	Intraven. Subcutan. Ewins

The numbers on the left-hand side of columns 3–13 (' Mgm. per 20 gm. mouse given intraperitoneally ') show the number mimals treated; those on the right-hand side show the result of treatment.

M.E.D. — Minimum effective dose. M.C.D. — Minimum curative dose. M.T.D. — Maximum tolerated dose.

0.00625 mgm. per 20 gm. mouse suffice to clear the peripheral blood in more than 50 per cent. of cases; permanent cures are obtained with doses of 0.01 and 0.0125 mgm., and with doses of 0.025 and 0.05 mgm. the great majority of animals are cured. The therapeutic index (Maximum tolerated dose) of 4:4'-diamidino stilbene is therefore about 30. Other compounds displaying exceptional activity are 4:4'-diamidino diphenoxy propane, R-O(CH₂)₂O-R, and

4: 4'-diamidino diphenoxy pentane, R-O(CH₂)₅O-R. With these, permanent cures were obtained in a considerable number of animals with a dose of 0.025 mgm. per 20 gm. mouse, and doses of 0.05 and 0.01 mgm. cured the great majority. As the maximum tolerated dose of these compounds is also approximately 1.0 mgm. per 20 gm. mouse, their therapeutic index is about 15.

Compounds R-O(CH₂)₂O-R and R-OCH₂O-R are apparently only slightly less active, with therapeutic indices of about 10, and these are followed closely

by R-O(CH₂)₂NH-R.

In the series $R\text{-}O(CH_2)_nO\text{-}R$ it is interesting to note that the trypanocidal activity steadily increases with the length of the alkane chain up to a point which is presumably reached when n=5 or therabouts; beyond this point the compounds become less active, until when n=10 relatively little activity is displayed.

Comparison of 4: 4'-diamidino diphenyl ether with the first four compounds in Group F shows that relatively slight changes in the radicle $\stackrel{\text{NH}}{\text{NH}_2}$ C completely destroy trypanocidal activity, at any rate when tested *in vivo*.

Another point of interest brought out in the table is that, in all cases in which the point was examined, the activity of the 4:4' diamidine compounds was distinctly greater than that of the 3:4' or 3:3' compounds.

TABLE II

Comparing the therapeutic activity of various aromatic arsenicals with that of the aromatic diamidines in mice infected with *T. rhodesiense*

Com	pounc	1		M.E.D.	M.C.D.	M.T.D.	no-regar	M.T.D.	M.T.D
				mgm	. per 20 gm. r		M.E.D.	M.C.D	
Atoxyl		* * *		2.0	5.0	10.0		.5	.)
Arsacetin				3.0	5.0	30.0		10	6
Tryparsamide				6.0	20.0	40.0		7	2
*Reduced arsaceti	n			0.025	0.1	0.25	,	10	2.5
Reduced tryparsa	mide t	thiogly	collate	0.04	0.5	1.5	-	4	3
Halarsol				0.02	0.1	0.6	1	30	6
Novarsenobillon				0.125	1.0	5.0	1	40	.5
R-OCH ₂ -R				0.0125	0.1	1.0	1	80	10
R-O(CH ₂) ₂ O-R				0.025	0.1	1.0	i	40	10
R-O(CH ₂) ₃ O-R				0.0125	0.05/0.1	1.0		80	15
R-O(CH ₂) ₅ O-R				0.01	0.05/0.1	1.0		100	15
R-CH: CH-R				0.00625	0.025/0.05	1.0		160	30

^{*} Reduced arsacetin = 4-acetylamino phenyl arsenoxide.

 $[\]uparrow$ Reduced tryparsamide thioglycollate = Disodium di-(carboxymethyl)-4-glycineamido phenyl thioarsinite.

M.E.D. = Minimum dose which suffices to clear the blood of parasites.

M.C.D. = Minimum dose necessary to produce permanent cures in about 80 per cent. of mice.

M.T.D. Maximum tolerated dose.

Table 111 Summarizing the results of treating rabbits infected with T, rhodesiense with various aromatic diamidines intravenously

		Compound R represents $NH > C$										
	Dose (mgm. per kilo.)	R-O(CH ₂) ₂ O-R	R-O(CI	H ₂) ₃ O-R	R-O(CI	H ₂) ₅ O-R	R-CH : CH-					
		No. of rabbits Result	No. of rabbits		No. of rabbits	Result	No. of rabbits	Result				
, % . draw	10.0				3	3C	2	2C				
lose	5.0				ŏ	2R 3C	ň	IR 4C				
Single dose	2.5				5	4R IC	6	2R 4C				
	1.25				5	4R IC	10	4R 6C				
***************************************	2·5 at 3- or 4-day intervals for 5 doses	3 2R 1C	ŏ	5C								
	1-25 on each of 10 consecutive days	5 IR 4C	5	5C								
	1·25 on each of 5 consecutive days		5	5C	ŏ	5(*	.)	5C				
Repeated doses	1·25 at 3- or 4- day intervals for 5 doses						ă	5C				
Repe	0.5 on each of 5 consecutive days		5	1R 4C	5	2R 3C	8	8C				
	0.5 at 3- or 4-day intervals for 5 doses		7	5R 2C			7	3R 4C				
	0.5 at 3- or 4-day intervals for 3 doses						5	4R IC				

R = Animal apparently recovered, but a relapse occurred.

C = Animal permanently cured,

The degree of therapeutic activity displayed by the most active of the compounds mentioned in Table I compares very favourably with those found by Yorke, Murgatroyd and Hawking (1931) in respect of various aromatic arsenicals.

The most active of these compounds, viz., R-CH: CH-R, R-O(CH₂)₅O-R, R-O(CH₂)₃O-R and R-O(CH₂)₂O-R, were then tested on rabbits in an advanced stage of infection with T. rhodesiense, with the results shown in Table III.

The maximum dose of these drugs tolerated by rabbits when administered intravenously is about 20 mgm. per kilo. of body weight. All the rabbits at the time of treatment had been infected between 3 and 4 weeks and exhibited pronounced lesions of the face and genitalia. It has previously been found that with the aromatic arsenicals, such as tryparsamide, it is necessary to give approximately the maximum tolerated dose in order to effect permanent cures by single doses in rabbits in such an advanced stage of the disease (Yorke and Murgatroyd, 1936). But in the case of the most active of these aromatic diamidines cures are obtainable with only about 1/4th of the maximum tolerated dose; thus 4 of 5 rabbits were cured by a single dose of 5.0 mgm. of R-CH: CH-R, and 3 of 5 rabbits by a similar dose of R-O(CH₂)₅O-R. The results obtained with a limited number of repeated doses were even more impressive. With the most active compound, R-CH: CH-R, such a minute dose as 0.5 mgm., i.e., 1/40th of the maximum tolerated dose, repeated on each of 5 consecutive days, cured all of 8 rabbits; and results only slightly less striking were given by R-O(CH₂)₃O-R and R-O(CH₂)₅O-R. All the rabbits were observed for at least 3 to 4 months after treatment.

Encouraged by the remarkable activity displayed by these aromatic diamidines in the treatment of mice and rabbits infected with our laboratory strain of *T. rhodesiense*, we decided to ascertain whether they had any action in infections of mice with *T. congolense* and *T. cruzi*.

Table IV shows that most of the aromatic diamidines under consideration have some action in *T. congolense* infections of mice, but only when given in large doses. A dose of 0·5 mgm. per 20 gm. mouse, i.e., about half the maximum tolerated dose, in most cases caused the trypanosomes to disappear from the peripheral blood, but relapses occurred in a few days. The only compound to produce any permanent cures when given in this dose was 4:4'-R-CH: CH-R; in smaller doses, viz., 0·1 and 0·25 mgm. per 20 gm. mouse, R-CH: CH-R failed to make the blood negative but prolonged life for several weeks beyond that of the control animals, which usually died in from 5 to 10 days. When given in the maximum tolerated dose (1·0 mgm. per 20 gm. mouse), 4:4'-R-CH: CH-R produced permanent cures in all of 7 mice treated, and 3:4'-R-CH: CH-R in 2 of 3 mice. From Table V, which gives the results obtained by smaller doses of 4:4'-R-CH: CH-R and 3:4'-R-CH: CH-R repeated on 3 or 5 consecutive days, it is seen that the results are much more

Table IV

Showing the results obtained by treating mice infected with T, congolense with single doses of the various aromatic diamidines

Compound NH	_			Dose, 1	ngm. p	er 20 gm.	mouse	intraperi	toneally	
R represents NH ₂			(0.1	()	0.25	()-5	1	.0
R-CH ₂ -R	•••		-	THE REST OF THE PARTY AND ADDRESS OF THE PARTY	***************************************	The state of the s	4	4N		
$R-(CH_2)_2-R$							4	4R		
t : +'- R-CH : CH-R	• • •	•••	-4	1N*	4	1N*	7	5R 2C	7	7C
3: 4'-R-CH: CH-R	* * *	• • •					:}	3R	3	IR 2C
1:4'-R-O-R									3	3R
4:4'-R-OCH ₂ -R							4	3N IR		
3 : 4'- R- OCH ₂ -R	• • •	• • •					3	3R	3	3R
R-CH ₂ OCH ₂ -R							1	4N		
R-OCH₂O-R							4	2N 2R		
R-O(CH ₂) ₂ O-R	• • •	• • •					:)	2N IR	3	3R
+ : 4'-R-O(CH ₂) ₃ O-R							3	3R	3	2R 1C
3 : 3'- R- O(CH ₉) ₃ O-R							*}	3R	3	1P 2R
R-O(CH ₂) ₅ O-R							3	3R	3	2P TR
$R-O(CH_2)_2NH-R$	• • •								3	3R

N = Blood not made negative.

 N^* = Blood not made negative, but life prolonged.

R = Blood became negative, but a relapse occurred,

C = Permanent cure.

P = Poisoned.

favourable. A dose of 0.25 mgm. repeated on each of 3 or 5 consecutive days sufficed to cure all the mice in the case of 4: 4'-R-CH: CH-R, but only a proportion in the case of 3: 4'-R-CH: CH-R.

The general conclusion from these observations appears to be that, whilst most of the compounds have a definite action in *T. congolense* infections, permanent cures are only occasionally obtained, except with 4:4'-R-CH: CH-R; this compound is curative when given in a single maximum tolerated dose or when 1/4th of the maximum tolerated dose is repeated on each of several consecutive days.

TABLE V
Showing the results obtained by treating mice infected with T. congolense by repeated doses of the most active diamidines

Compound		Dose, mgm. per 20 gm. mouse intraperitoneally										
R represents $\underset{\text{NH}_2}{\text{NH}} \subset \langle$			each of 5 utive days		each of 3 itive days		n each of a					
4 : 4 '-R-CH : CH-R		5	5R	9	9C	4	4C					
3 : 4'-R-CH : CH-R	• • •			5	3R	4	1R					
					2C		3C					

R = Blood became negative, but a relapse occurred.

C = Permanent cure.

A number of the aromatic diamidines, viz., R-O-R, R-O(CH₂)₂O-R, R-O(CH₂)₃O-R, R-O(CH₂)₅O-R, R-O(CH₂)₂NH-R, 4:4'-R-CH: CH-R and 3:4'-R-CH: CH-R, were tested on T. cruzi infections of mice. Maximum tolerated doses of 1 mgm. per 20 gm. mouse were given on two occasions at an interval of 2 or 3 days. These had no obvious effect, and the number of trypanosomes in the peripheral blood was not influenced.

From these experiments it seemed clear that the compounds exert no action on the flagellate forms of the parasite found in the peripheral blood, but it did not necessarily follow that they were without action on the Leishmania forms in the heart and skeletal muscles. With a view to investigating this point, a dose of 0.25 mgm. of 4: 4'-R-CH: CH-R was given on 10 consecutive days to 10 mice infected with *T. cruzi*. In all 10 mice the infection persisted, as it did in the control animals, throughout this course of treatment. Three mice died, apparently poisoned, on the last day of treatment and on the day afterwards; in the remaining 7 the infection continued as in the control animals, and parasites were found in the heart-muscle of the mice, which were killed some weeks after the conclusion of the treatment.

It appears, therefore, that these aromatic diamidines have no action on either the flagellate or the Leishmania forms of *T. cruzi*.

Whether these compounds will have any value in the treatment of human sleeping sickness and of trypanosomiasis of stock remains to be seen. The most active (R-CH: CH-R, R-O(CH₂)₃O-R and R-O(CH₂)₅O-R) are at present being tested in the field; the preliminary reports suggest that they have a pronounced action, at least in early cases of human trypanosomiasis, but it is still too early to formulate any definite opinion regarding their practical value.

It might here be remarked that none of the compounds exerted any appreciable action on *Spirochaeta recurrentis* or on *Spirillum minus* infections in mice. Some of them, however, exhibited a remarkable activity against certain other protozoal infections, notably those due to Leishmania and Babesia, but this is a matter which will be discussed in later papers of this series.

In conclusion, reference should be made to the toxic effect of these aromatic diamidines observed in various animals and in man.

Mice. As already mentioned, the maximum dose tolerated by mice when administered intraperitoneally is, in almost all cases, about 1 mgm. per 20 gm. mouse (50 mgm. per kilo.), but with this dose occasional deaths are recorded. Doses of 2 mgm. and more per 20 gm. mouse were almost invariably fatal; toxic symptoms consisting of narcosis, dypsnoea, tremors and convulsions appeared within a few minutes, and death often resulted within an hour. Fractional doses of 0·1 mgm. per 20 gm. mouse can be administered daily for prolonged periods without producing any signs of intoxication, and doses of 0·25 mgm. per 20 gm. mouse can be given for at least 7 days, and often longer, without any apparent ill effect.

Rabbits. The maximum dose of the most powerfully trypanocidal of these substances, viz., R-CH: CH-R, R-O(CH₂)₃O-R and R-O(CH₂)₅O-R, tolerated by rabbits when given intravenously is about 20 mgm. per kilo. of body weight. Doses of 30 mgm. or more are almost invariably fatal. After doses of about 20 mgm. per kilo. there is frequently immediate collapse, with evidence of respiratory and circulatory failure, but within a few minutes the animal usually recovers from the shock and is apparently none the worse; still larger doses produce profound shock-collapse, often accompanied by diarrhoea, and death follows within an hour.

Cats. Dr. Wien has reported on some of the physiological effects of $R-CH_2O-R$ and $R-O(CH_2)_2O-R$ on cats anaesthetized with chloralose. He found that doses of 5 mgm. and 10 mgm. per kilo. produced a big fall in blood-pressure, with recovery to the original level within a few minutes. With the smaller dose there was no obvious effect on the heart volume, but after the larger dose there was a slight increase in volume and diminution in amplitude; the effect on the heart passed off on recovery of the blood-pressure. A dose of 5 mgm. per kilo. of $R-O(CH_2)_2O-R$ produced a considerable effect on the respiration, which was

at first depressed, followed by gasping, and later was much deeper; the rate was hardly altered.

Dogs. The maximum dose of these compounds tolerated by puppies when administered subcutaneously varies considerably. A dose of 10 mgm. per kilo. of R-CH: CH-R is usually fatal, but 5 mgm. per kilo. is tolerated; R-O(CH₂)₃O-R and R-O-R. are less toxic, the animal surviving doses of 15 mgm. per kilo. in the case of the former and 20 mgm. per kilo. in that of the latter.

Even after moderate-sized doses, dogs often vomit and defaecate, and sometimes there is swelling of the face and transient weakness and tremors, but these signs disappear within an hour. With larger doses, bordering on the lethal, these signs may be accompanied by collapse, due presumably to respiratory and circulatory disturbances: if the animal recovers from these immediate effects, a peculiar spastic paresis may develop 3 to 4 days later, and, when once this condition appears, the end is almost invariably fatal. As is well known, puppies are particularly liable to develop intussusception as the result of any intestinal irritation, such as infestation with intestinal parasites. This condition was encountered from time to time in young puppies treated with the aromatic diamidines, and was apparently due to intestinal contractions produced by the compounds.

Cattle and sheep. Hitherto we have had little experience of the use of the aromatic diamidines in these animals, but there seems reason to believe that they will tolerate larger amounts than dogs.

Man. The only compounds which have been administered to man up to the present are R-CH: CH-R and R-O(CH₂)₅O-R. They have been administered both intramuscularly and intravenously to a considerable number of individuals, in doses varying from 0.5 mgm. to 2.0 mgm. per kilo. of body weight, and the doses have been repeated either daily, on alternate days or twice weekly, until a maximum of 10 have been given. No accident has so far occurred, but many of the patients who have received the larger doses (1.0 and 2.0 mgm. per kilo.) by the intravenous route have exhibited transient symptoms. These varied from flushing of the face and indefinite epigastric sensation to headache, rapid pulse, sweating, retching and occasionally vomiting. In all cases these signs, which are probably the result of stimulation of the parasympathetic nervous system, passed off within half an hour, and the patient felt no worse for the experience. There is some reason to believe that these immediate transient disturbances are no longer observed after the first two or three doses. In the doses given, the compounds did not produce albuminuria or other signs of renal irritation.

SUMMARY

In previous papers it was shown that certain guanidines, isothioureas, amidines and amines with alkyl and alkylene chains exhibited considerable trypanocidal activity. As it seemed possible that the carbon chain merely served

as a carrier of the active terminal groups of strong basic nature, a number of aromatic amidine and guanidine compounds were prepared; they also were found to exhibit trypanocidal action.

Dr. Ewins, of Messrs. May and Baker, Ltd., with the object of developing this line of investigation, prepared a large number of aromatic compounds containing the amidine group, and preliminary tests on mice showed that many of

these compounds were powerfully trypanocidal.

The present investigation is concerned with a more extensive examination of the most active of these compounds. The most important, from the trypanocidal point of view, were members of the series $4:4'-R-O(CH_2)_nO-R$, where R is NH > C, and 4:4'-R-CH:CH-R. Certain members of the series $4:4'-R-O(CH_2)_nO-R$, notably $R-O(CH_2)_5O-R$ (diamidino diphenoxy pentane) and $R-O(CH_2)_3O-R$ (diamidino diphenoxy propane), and 4:4'-R-CH:CH-R (diamidino stilbene), exhibited a truly remarkable trypanocidal effect on mice infected with our laboratory strain of T. rhodesiense. When administered intraperitoneally the maximum dose tolerated by mice is in each case about 1 mgm. per 20 gm. mouse. With the most active compound, 4:4'-R-CH:CH-R, such minute doses as 0.005 and 0.00625 mgm. per 20 gm. mouse suffice to clear the peripheral blood of trypanosomes in more than 50 per cent. of cases; permanent cures are obtained with doses of 0.01 and 0.0125 mgm., and with doses of 0.025 and 0.05 mgm. the great majority of animals are cured. The therapeutic index (Maximum tolerated dose) of 4:4'-diamidino stilbene is

The therapeutic index (Maximum tolerated dose Minimum curative dose) of 4:4'-diamidino stilbene is

accordingly about 30. The other two compounds, viz., $4:4'-R-O(CH_2)_5$ O-R and $4:4'-R-O(CH_2)_3$ O-R, are only slightly less active. This degree of trypanocidal activity surpasses that of any of the aromatic arsenicals.

Tests on rabbits in an advanced stage of infection with the same trypanosome gave equally striking results. The maximum dose tolerated by rabbits when treated intravenously is about 20 mgm. per kilo. With 4: 4'-R-CH: CH-R, single doses of 1.25 to 5 mgm. per kilo. cured the majority of animals, and repeated small doses gave even better results, 0.5 mgm. (i.e., 1/40th of the maximum tolerated dose) repeated on each of 5 consecutive days curing all of 8 rabbits. The results obtained with the other two compounds are nearly as good.

While many of the compounds displayed some action on *T. congolense* infections in mice, cures were obtained only with 4:4'-R-CH: CH-R, and then only when the drug was given in maximum doses; all of 7 mice were cured by a single dose of 1.0 mgm., and 2 of 7 by a dose of 0.5 mgm. Repeated fractional doses gave better results, all of 9 mice being cured by 0.25 mgm. given on each of 3 consecutive days.

None of the compounds had any action on T. cruzi infections of mice, nor on infections due to Spirochaeta recurrentis or Spirillum minus. Certain of them,

however, exhibit a remarkable activity against other protozoal infections, notably those due to Leishmania and Babesia, but this will be discussed in subsequent

Some description is given of the toxic manifestations exhibited by various animals and by man after the administration of relatively large doses of these aromatic diamidines.

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STUDIES IN CHEMOTHERAPY*

XXII.—THE ACTION OF CERTAIN AROMATIC DIAMIDINES ON BABESIA CANIS INFECTIONS OF PUPPIES

BY

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Since Nuttall (1909) drew attention to the therapeutic activity of trypan blue in Babesia infections, many attempts have been made to find a more efficient remedy. Apart, however, from the discovery of Stephan and Esquibel (1929) that acriflavine also exerted a definite action on certain Babesia infections, there seems to have been no real advance in the therapy of this group of diseases until 1935, when Kikuth (1935) and Cernaianu and others (1935) published accounts of the very successful results obtained with a Bayer product known as acaprin (N,N'-dimethylquinolyliummethyl sulphate-6-urea) in the treatment of various Babesia infections.

Two or three years ago, when we were working with the aliphatic diamidines, we observed that n. undecane diamidine exerted a definite action in *Babesia canis* infections of puppies, but that cures could not be obtained with this compound. The observation encouraged us to examine whether the more powerfully trypanocidal aromatic diamidines prepared by Dr. Ewins (Lourie and Yorke, 1939) exerted any action on *Babesia canis*. The strain used by us was obtained from Dr. Kikuth, of Elberfeld, and is very virulent for young puppies, killing them as a rule in from 4 to 6 days. The results obtained with a number of Dr. Ewins' compounds are summarized in Table I. In these experiments young puppies were used, and the drugs were injected subcutaneously about 24 hours after the parasites had appeared in the peripheral blood. This was necessary as the disease in the untreated animals develops with such rapidity that the puppies die as a rule within a couple of days of the first appearance of parasites in the blood.

^{*}This work was assisted by grants from the Medical Research Council and from Messrs. May and Baker Ltd.

Showing the results of treating puppies infected with Babesia canis with single doses of various aromatic diamidines given subcutaneously

							000					
	0.25	C C Sprane Design of the					A drawn and a second					5 4R
	1.0		a IR									5 1P
-	1.5		9 4R 5C				Company of the compan					
ly weigh	5.5		5 2R 3C	4 3R 1C	- IR	_ Z		3 3R	oi NE	- IR		3 1P
o. of boc	ī.	- IR	6 1P 5C	6 4C 4C	5. 5. R. 5.	2 2R	1 1P	5 5C	2 2R	51 FF		2 2p
n. per kil	10	- - 	5 ± P	5 5C			1 1P		- R	<u>=</u>		Application of the state of the
Dose, mgm. per kilo. of body weight	15	- 18	2 2p				- L	es Se Se			- 18	
D	05		2 2p	5 5 5 S				4 3P		The second secon		
	25			ss ss								
	30			51 F E		The state of the s		I IP				
NHW.	NH ₂	4 : f'-diamidino diphenyl methane	4 : 4'-diamidino stilbene	1: 4'-diamidino diphenyl ether	4: 4'-diamidino phenyl benzyl ether	4: 4'-diamidino diphenoxy methane	4:4'-diamidino diphenoxy ethane	4:4'-diamidino diphenoxy propane	4: 4'-diamidino diphenoxy pentane	4:4'-diamidino β -phenoxy ethyl aniline	4 : 4'-diamidino dibenzyl ether	
NH N	N ichicachta	4:4'-R-CH ₂ -R	4:4'-R-CH:CH-R	4: 4'-R-O-R	4:4'-R-CH ₂ O-R	4:4'-R-OCH ₂ O-R	4:4'-R-O(CH ₂) ₂ O-R	4: 4'-R-O(CH ₂) ₃ O-R	4:4'-R-O(CH ₉) ₅ O-R	4:4'-R-O(CH2)2NH-R	4: 4'-R-CH ₂ OCH ₂ -R	Acaprin

These diamidines were in all cases used in the form of their dihydrochlorides, to which the figures in the tables actually refer.

The numbers on the left-hand side of columns 3-12 ('Dose, mgm. per kilo. of body weight') show the number of animals treated; those on the right-hand side show the result of treatment.

P = Poisoned.

R = Blood became negative, but a relapse occurred.

C = Cured.

S = Survived (uninfected animals).

The information contained in Table I shows that almost all the compounds tested exert some influence on the infection, in that the blood became negative after the injection. Only three of the compounds, however, exerted a definite curative effect: these were diamidino stilbene (R-CH: CH-R), diamidino diphenvl ether (R-O-R), and diamidino diphenoxy propane (R-O(CH₂)₃O-R). The most active of the three compounds appears to be R-CH: CH-R, as 3 of 5 puppies were cured by a single dose of 2.5 mgm. per kilo. of body weight, and 5 of 6 puppies with a single dose of 5.0 mgm. per kilo. Unfortunately, however, R-CH: CH-R appears to be unusually toxic for dogs, which will not tolerate more than 5 mgm. per kilo., and even this dose is occasionally fatal. R-O(CH₂)₃O-R in a dose of 5 mgm. per kilo, cured all of 5 puppies; this compound is less toxic for puppies than R-CH: CH-R and appears to be tolerated in doses up to 15 mgm. per kilo., i.e., about three times the curative dose. R-O-R in a dose of 5 mgm. per kilo, cured 4 of 6 puppies, and, in a dose of 10 mgm., all of 5 puppies; this compound is apparently distinctly less toxic for puppies than the other two, and is tolerated in doses of 20 to 25 mgm. per kilo.

For comparison, we have given at the foot of the table a number of observations made with Bayer's preparation acaprin. The dose recommended for the

Table II

Showing the results of treating puppies infected with *Babesia canis* with two small doses of aromatic diamidines given subcutaneously on successive days

		Therature		Compound						
		1 reatm	ent, mgm. p	er Kilo.	R-CH	: CH-R	R-O(CH ₂) ₃ O			
1.5 mgm, on each of two consecutive days					 7	3R 4C	11	8R 3C		
2.5		The second secon	ngan nin nga samatanan na anao ipinaha manamanan mataba. B	1.5		The second second	6	6C		

The numbers on the left-hand side of columns 2 and 3 ('Compound') show the number of animals treated; those on the right-hand side show the result of treatment.

C = Cured.R = Blood became negative, but a relapse occurred.

treatment of dogs suffering from *Babesia canis* is 0.25 mgm. per kilo. With this dose we succeeded in curing only 1 of 5 puppies; with a dose of 1.0 mgm. per kilo. 4 of 5 animals were cured, and 1 was poisoned; with a dose of 2.5 mgm. 1 of 3 puppies was poisoned and the 2 which recovered were profoundly affected by the drug; a dose of 5 mgm. per kilo. killed both of the animals to which it was given. Under the conditions of our experiments, 1.0 mgm. per kilo. appears to be the curative dose of acaprin; this is distinctly smaller than the curative dose of the present compounds, but against this is the fact that acaprin is toxic

for the host in much smaller doses; the maximum tolerated dose for puppies is probably from 1.0 to 2.5 mgm. per kilo., and for mice it is 0.1 mgm. per 20 gm. mouse, as compared with about 1.0 mgm. for the diamidines.

The effect of treating infected puppies with two small doses of 4:4'-R-CH: CH-R and 4:4'-R-O(CH₂)₃O-R was examined, and the results obtained are summarized in Table II, from which it is seen that a dose of 2.5 mgm. per kilo. of 4:4'-R-O(CH₂)₃O-R given on each of two consecutive days sufficed to cure all of 6 puppies; a dose of 1.5 mgm. of this drug or of R-CH: CH-R also given on each of two consecutive days failed, however, to cure more than a proportion of the animals.

It is, of course, well known that, although young puppies infected with Babesia canis develop an acute disease from which they usually die within a few days, occasionally they survive the acute attack and pass into a chronic stage with scanty parasites in the blood; they may eventually die of the disease some weeks later, either as the result of exhaustion or from an acute exacerbation, or they may gradually obtain the upper hand and recover completely. In contrast, older dogs frequently recover from the acute attack and pass into a chronic stage of the disease, from which they usually recover.

During the chronic stage parasites cannot be found by direct blood examination, but their presence can be revealed by inoculation of the blood into young puppies. How long this state of balance or tolerance lasts is unknown, but it certainly persists for many months, and probably for a year or more. There is no reason to doubt that such animals can infect the invertebrate host, and that consequently they constitute a reservoir of the virus which is responsible for new infections.

As will be seen from Tables I and II, many of the puppies treated with the less active compounds, or with the smaller doses of the more active compounds, are recorded as having relapsed. In these cases parasites reappeared in the peripheral blood after periods varying from 6 to 14 days. The fate of such animals varied; because they were almost all young puppies they exhibited little resistance to the infection, and usually died either from an acute exacerbation or from exhaustion resulting from a subacute infection. A few of them, however, passed into the chronic condition referred to above, in which parasites could not be found in the blood or only in very scanty numbers at rare intervals.

In our experience, treated animals which relapsed did so in every instance, except one, within 14 days. In the exception, which had received 2 doses of 1.5 mgm. of R-O(CH₂)₃O-R on consecutive days, parasites did not reappear in the blood until 33 days after treatment. In none of our animals did a relapse occur after this period, although many of them were kept under daily observation for up to three months after treatment. Strong support for the view that these animals were really sterilized of the infection is obtained from the fact that in many cases their blood was inoculated into young puppies and in no instance produced infection. This is in striking contrast to the state of affairs seen in

the case of older dogs which had apparently recovered spontaneously from the disease, but whose blood remained infective for puppies for many months after it had become impossible to find parasites by direct examination of the blood and after all signs of disease had disappeared.

There is, therefore, strong evidence that we had succeeded in producing a true sterilisans magna by treatment of acutely infected puppies by these compounds, and that they were actually cured and not merely brought into a state of tolerance comparable with the natural spontaneous recovery occasionally seen in young puppies but much more frequently in older dogs. In this connection, it is interesting to note that reinfection of a spontaneously recovered animal, in a stage of balance or tolerance, fails to produce a fresh acute attack, whereas reinfection of animals which have been actually cured by the administration of one of the diamidines produces an acute and fatal attack. The spontaneously recovered animal in a state of tolerance is therefore immune to further infection with the homologous strain of parasites, whereas the animal actually cured by means of the drug is susceptible to reinfection.

Attempts to produce cures by treating relapses which occurred after the administration of subcurative doses of the diamidines were sometimes successful, but often they failed. This work led to an unexpected discovery, viz., that the parasite rapidly develops resistance to the drug. This is well shown by the following observations:—

Puppy 14 1939

```
Inoculated with normal strain of Babesia canis.
June
           Blood contained 20 parasites per field. R-CH: CH-R,
                1.5 mgm. per kilo.
       6th-11th Blood negative.
      12th 1 parasite in 20 fields.
                        20
                                   R-CH: CH-R, 1.5 mgm. per kilo.
      13th
                                   R-CH: CH-R, 1.5
           Blood negative.
      14th
      15th
            I parasite in 10 fields.
      16th
                         5
      17th
                  2.2
                         2
      18th
                  53
      19th
           1
                         5
                             2.3
                         2
            1
                                   R-CH: CH-R, 1.5 mgm. per kilo.
      21st
                             2.9
      22nd 1
                        20
                                   R-CH: CH-R, 1.5
      23rd 1
                        100
                             2.7
 2.2
      24th 1
                         40
      26th 1
                         1
      27th 1
                         1
                        30
      30th
           1
                                   Subinoculated into puppy 132.
July
       3rd
           1
                        50
                  ,,
                        50
       7th
           1
                        20
      11th
           1
      14th Blood negative.
                                   Subinoculated into puppy 166.
      15th
            1 parasite in 50 fields.
      17th
           Animal killed.
     26th
```

```
Puppy 132
    1939
               30th Inoculated from puppy 14.
         June
                                               R-CH: CH-R, 1.5 mgm. per kilo.
                4th 3 parasites in 1 field.
         July
                 5th 10
                                               R-CH : CH-R, 1.5
                                        2.3
           33
                6th 5
                                    1
          2.9
                 7th 8
                                    1
           "
                             2.2
                                        2.5
                8th 3
                                    1
           23
                                        99
                9th 3
                             22
          23
                10th 3
                                    1
          25
                11th 10
                                    1
                12th-26th
                            Disease ran a subacute course and animal died on
                           July 26th with a heavy infection
PUPPY 166
    1939
                      Inoculated from puppy 14.
         July
                15th
                19th
                      1 parasite in 100 fields.
                20th 20
                                               R-CH: CH-R, 2.5 mgm. per kilo.
                                    1
                21st
                                     3
                                               R-CH: CH-R, 2.5
                      1
                            . .
                                         2.2
                22nd 1
                                   200
                             2.3
                                         9.9
                23rd 1
                                     1
                             2.2
                                         13
                                               R-CH: CH-R, 2.5 mgm. per kilo.
                24th 1
                                     1
                             9.9
                                         .
                                               R-CH: CH-R, 2·5
R-CH: CH-R, 2·5
                25th 1
                                     1
                                         9.1
                             1.0
                      2
                                     1
                26th
                                         3 4
                             1.3
                27th
                                     10
                      1
                             13
                                     40
                28th
                      1
                             2.2
                29th 3
                                      1
                                               Subinoculated into puppy 186.
                30th onwards The disease ran a chronic course.
PUPPY 186
    1939
               29th Inoculated from puppy 166.
                                               R-CH: CH-R, 5 mgm. per kilo.
                      2 parasites in 1 field.
         August 1st
                 2nd 2
                                    1
           ,,
                 3rd 2
                                    1
                             33
           2.3
                 4th 5
                                    1
           2.2
                 5th 10
                                    1
                                               Subinoculated into adult dog 199.
                                        2.2
                 6th 1
                 7th-19th
                            Disease ran a subacute course and the puppy died on
                           August 19th with a heavy infection.
Dog 199
    1939
         August 5th Inoculated from puppy 186.
                 8th 1 parasite in 200 fields.
                                     1
                 9th
                                          **
                10th 30
                                          11
                             . .
            ,,
                11th 5
                                     1
                                          11
                             2.3
            3.5
                                     20
                12th
                      1
                             22
            3.1
                14th
```

The strain was made resistant in puppy 14 by administering a subcutaneous dose (1.5 mgm.) of R-CH: CH-R, and by repeating this dose on each of two consecutive days when a relapse occurred 8 days after the initial dose. It is

to be noted that even at this early stage in the proceedings the parasite exhibited signs of resistance in that the double dose cleared the blood for only 2 days, whilst the original dose cleared it for 6 days. Two more similar doses of the drug given 8 and 9 days later produced no effect on the infection, so that at this point there seemed clear evidence of definite resistance.

On June 30th and July 15th the strain was inoculated into puppy 132 and puppy 166 respectively. It is seen that in puppy 132 the parasite was resistant to a double dose of 1.5 mgm., and in puppy 166 to a double dose of 2.5 mgm. On July 24th, 25th and 26th, puppy 166 was given 3 doses of 2.5 mgm. per kilo., and the infection proved completely resistant to what in the ordinary way would be an almost certainly curative course of treatment.

On July 29th, the strain was transferred from puppy 166 to puppy 186, and in this animal was found to resist completely the curative dose of 5 mgm. per kilo.

It appears, therefore, that we have here to deal with the development of true drug-resistance in *Babesia canis*, which is considerable in degree and remains unchanged when the strain is passaged through a series of puppies. This drug-resistance is remarkable for the speed with which it develops.

The fact that *Babesia canis* rapidly develops resistance to the aromatic diamidines after the administration of a few subcurative doses of one of them indicates that it is hopeless to attempt to sterilize the infection by treatment of relapses with subcurative doses. Such doses very soon cease to have any effect. This is in striking contrast to what is seen in the treatment of malaria by quinine. As a rule, quinine, in whatever dosage, or by whatever method it is given, fails to sterilize a malaria infection, and the usual method of treatment consists in controlling the infection by administering the drug over prolonged periods, either continuously, or at weekends, or by treating relapses until eventually the patient cures himself. No one has, as yet, produced any convincing evidence that the malaria parasites develop any resistance to quinine.

SUMMARY

1. Most of the aromatic diamidine compounds examined had a definite action on *Babesia canis* infections of puppies in that they caused the blood to become negative for a time.

2. The most active compounds were diamidino stilbene (R-CH: CH-R), diamidino diphenyl ether (R-O-R), and diamidino diphenoxy propane (R-O(CH₂)₃O-R). Permanent cures could be obtained with single doses of these substances or with two smaller doses given on successive days.

3. Babesia canis could readily be made resistant to these aromatic diamidines, and the drug-resistance persisted unchanged after passage of the parasite through a number of puppies.

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THE ACTION OF 4:4'-DIAMIDINO STILBENE ON LEISHMANIA DONOVANI IN THE SYRIAN HAMSTER CRICETUS AURATUS

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(Received for publication August 10th, 1939)

Professor Warrington Yorke, F.R.S., to whom we express our thanks, kindly forwarded us samples of 4:4'-diamidino stilbene (see Lourie and Yorke, 1939), with a suggestion that we should try the drug on leishmaniasis and other protozoal infections. For this purpose we used Syrian hamsters infected with an Indian strain of *L. donovani*, which we obtained from Professor B. M. Das Gupta, of the Calcutta School of Tropical Medicine. The hamsters were taken from a series inoculated as follows with flagellates from cultures on Locke-blood-agar:

Oct. 12th, 1938—Each animal inoculated with 1 c.cm. culture intraperitoneally.

On December 1st, 1938, a number of animals were laparotomized, fragments of spleen were removed and found to be slightly infected.

On April 5th, 1939, 12 hamsters were laparotomized and fragments of spleen were removed for smears and histological examination. From this series, eight hamsters were taken for trials of the drug. Although the animals were taken from the same stock, kept on the same diet under identical conditions, and inoculated simultaneously with the same strain of *L. donovani*, they showed a very considerable variation in the degree of infection. Since it is difficult to make a quantitative estimate of the degree of general infection with visceral leishmaniasis, we adopted the following arbitrary method: several hundred nuclei of spleen cells of all types were counted, and the corresponding total number of definite L.D. bodies were estimated in a smear. The result is indicated below for each animal as the number of L.D. bodies per 100 nuclei. In the present series this number varied from 6 to 300. This rough estimate of the splenic infection conformed with subsequent histological examinations.

The intensity of the splenic infection in man and in the hamster is a good indication of the degree of the general infection, but this is not the case in the dog, where a heavy spleen infection may be accompanied by a slight infection in the skin and mucous membranes of the mouth, and vice versa. The effect of a chemotherapeutic agent in dogs can be estimated by changes in the clinical condition, by histological examination of fragments of skin, and cultures of puncture material from the popliteal gland, spleen, liver or sternum. In the Syrian hamster a change in the number of parasites in the spleen is a good index of therapeutic action, for an infection once established never regresses spontaneously (as is not infrequently the case in dogs under the influence of a good diet).

TOXICITY OF 4:4'-DIAMIDINO STILBENE

A single injection of 120 mgm. per kilo. body weight killed an animal in 48 hours. On the second day after the injection the animal crawled slowly and was unable to walk. When placed on his side, he did not attempt to change his position spontaneously. Histological examination showed damage to the kidneys, very extensive desquamation of cells in the tubules—particularly the convoluted tubules—and widening of the capillaries of the glomeruli. The liver was congested, and showed a few irregular patches of fatty degeneration.

Four injections each of 70 mgm. per kilo. on alternate days were fatal. Twelve injections each of 40 mgm. on alternate days produced no ill effects.

TREATMENT

Injections were made intraperitoneally on alternate days, except where otherwise indicated. At various intervals the animals were laparotomized, fragments of spleen were removed for histology and for smears, and splenic juice was sown on Locke-blood-agar. (The latter is the most sensitive of all media which we have tried, and can detect minute quantities of parasites.) The details and results are given below.

HAMSTER No. 1

Number of L.D. bodies per 100 nuclei: 250.

Dose per injection: 2.5 mgm. per kilo. body weight.

Laparotomized after 7 injections; no change in number of L.D. bodies. Died after 17 injections, cause unknown. Number of L.D. bodies: 100 per 100 nuclei.

Hamster No. 2

Number of L.D. bodies per 100 nuclei: 20.

Dose per injection: 2.5 mgm. per kilo. body weight.

Laparotomized after 19 injections; 1 L.D. body per 200 nuclei. Cultures positive.

Laparotomized after 27 injections; L.D. bodies: 0. Cultures negative during observation-period of three weeks.

Died after 28th injection of post-operative peritonitis.

HAMSTER No. 3

Number of L.D. bodies per 100 nuclei: 25.

Dose per injection: 5 mgm. per kilo. body weight.

Laparotomized after 18 injections; L.D. bodies: 0. Cultures positive.

Died after 25 injections of post-operative peritonitis.

Hamster No. 4

Number of L.D. bodies per 100 nuclei: 120.

Dose per injection: 10 mgm. per kilo. body weight.

Laparotomized after 8 injections; number of L.D. bodies per 100 nuclei: 20.

Laparotomized after 15 injections; number of L.D. bodies per 100 nuclei: 0. Cultures positive.

Laparotomized after 24 injections; cultures negative during observationperiod of three weeks.

HAMSTER No. 5

Number of L.D. bodies per 100 nuclei: 8.

Dose per injection: 20 mgm. per kilo. body weight.

Laparotomized after 10 injections; number of L.D. bodies per 100 nuclei: 0.

Died of pneumonia after 18 injections.

Hamster No. 6

Number of L.D. bodies per 100 nuclei: 75.

Dose per injection: 20 mgm. per kilo. body weight.

Laparotomized after 4 injections; number of L.D. bodies per 100 nuclei: 16.

Laparotomized after 10 injections; number of L.D. bodies per 100 nuclei:

0. Cultures negative during observation-period of three weeks.

HAMSTER No. 7

Number of L.D. bodies per 100 nuclei: 180.

Dose per injection: 40 mgm. per kilo. body weight.

Laparotomized after 3 injections; number of L.D. bodies unchanged.

Laparotomized after 6 injections; number of L.D. bodies per 100 nuclei: 70.

Between the 6th and 7th injections there was an interval of 5 days.

Laparotomized 1 day after 7th injection; no normal L.D. bodies were seen, but several masses of the shape and size of L.D. bodies with a nucleus, some without a parabasal, and others with minute faintly staining granules in place of a parabasal, were found.

Died of pneumonia after 12 injections. Number of L.D. bodies found: 0.

HAMSTER No. 8

Number of L.D. bodies per 100 nuclei: 6.

Dose per injection: 40 mgm. per kilo. body weight.

Laparotomized after 3 injections and died under anaesthetic; number of L.D. bodies per 100 nuclei: 2.

In addition to the above eight animals treated with 4:4'-diamidino stilbene, three animals were treated with urea stibamine. The details are as follows:

HAMSTER No. 9

Number of L.D. bodies per 100 nuclei: 70.

Dose per injection: 8.3 mgm. per kilo. body weight.

Died after 5 injections. No change in number of L.D. bodies.

Hamster No. 10

Number of L.D. bodies per 100 nuclei: 300.

Dose per injection: 12.5 mgm. per kilo. body weight.

Laparotomized after 5 injections; number of L.D. bodies per 100 nuclei: 150.

Laparotomized after 14 injections; number of L.D. bodies found: 0.

HAMSTER No. 11

Number of L.D. bodies per 100 nuclei: 20.

Dose per injection: 20 mgm. per kilo. body weight.

Laparotomized after 10 injections; number of L.D. bodies per 100 nuclei:

1. Cultures positive.

Laparotomized after 18 injections; number of L.D. bodies found: 0. Cultures positive.

HISTOLOGICAL EXAMINATION

Histological examinations were in satisfactory harmony with the findings in spleen smears with regard to intensity of infection. In assessing the histological changes in the spleen during the whole course of treatment, we have to bear in mind that the organ was subjected to repeated trauma at each laparotomy, and as a result was adherent both to the neighbouring viscera and to the abdominal wall, and it is difficult to dissociate the effects of the operations from those of the drug.

With this reservation, we give the following brief description of the changes observed, which were fairly uniform in all the animals in which the infection, as far as could be determined by microscopical examination, was cleared up. This subject remains to be studied in detail in future experiments, because up to the present nothing is known of the histological changes in a spleen, human or animal, which, as a result of treatment, is reduced from three or more times

to normal size. It is not even known whether the spleen in a cured case really becomes normal in structure.

In the spleen of an infected hamster with a degree of infection within the range of that found in the majority of the animals in the above series of experiments, the following picture is found.

The spleen is enlarged roughly to about four times the normal or more. The Malpighian follicles are enlarged and their boundaries irregular. The red pulp is congested and extremely cellular, containing, in addition to numerous nuclei of reticular cells, lymphocytes, large mononuclears and a varying number of plasma cells. A very striking feature in the spleen at this stage is the relatively large number of megakarvocytes (Plate IV, fig. 1) situated mainly near the periphery of the follicles on the irregular border between the white and red pulp. (In the spleen of normal hamsters megakaryocytes are few.) At the time the infection is cleaned out (though cultures may be positive), the above picture has not changed much, except that the megakaryocytes have disappeared (Plate IV, fig. 2). The red pulp appears to be slightly less cellular, though still congested. We did not stop treatment or laparotomy at this stage, and therefore cannot say how far the ensuing and more striking changes are due to this fact, and how far they are spontaneous. Eight to twenty days after the above stages (hamsters 2, 3, 4, 6, 7, 11, of which nos. 4, 6 and 11 were not complicated by secondary infections of any kind), the spleen was still enlarged, its consistency firm, the follicles atrophied, and the most striking changes occurred in the red pulp. The congestion has completely disappeared, the number of free cells has diminished, none or few plasma cells are found, and, as compared to the first and second stages, the red pulp is strikingly less cellular, particularly round the sinuses, and contains a larger proportion of connective tissue (Plate V, fig. 2).

REMARKS

The above experiments clearly prove that 4:4'-diamidino stilbene has a definite chemotherapeutic action on *Leishmania donovani* in Syrian hamsters, and in three animals sufficiently investigated (nos. 3, 4 and 6) there was, as far as could reasonably be determined, a complete disappearance of the infection from the spleen. This is the first instance of a drug not containing antimony with a marked chemotherapeutic action on an infection of *Leishmania donovani*. This therapeutic action is indisputable, because, as previously stated, infections of *L. donovani* in the Syrian hamster never regress spontaneously.

Beyond this, no conclusions are drawn for the present, because the number of animals observed is too small to eliminate individual variations, but there are the following indications which remain to be investigated in experiments on a larger scale.

1. The amount of drug required to eradicate an infection depends on the intensity of the infection. The relatively heavy infection of hamster no. 1 was reduced but was still considerable (100 L.D. bodies per 100 nuclei) after 17

injections, each of 2.5 mgm. per kilo. body weight, while the milder infection of hamster no. 2 almost disappeared after 19 injections of the same dose.

2. The therapeutic effect depends not only on the total amount of drug injected, but also on the time-distribution of the total amount, e.g., 18 injections each of 5 mgm. per kilo. in hamster no. 3 were not as effective as 27 injections of 2.5 mgm. per kilo. in hamster no. 2; 3 injections of 40 mgm. per kilo. in the relatively slight infection of hamster no. 8 were not nearly as effective as smaller quantities of the drug distributed over a longer period in the much heavier infections of hamsters nos. 2 and 3 (all injections at intervals of two days).

The optimum dosage and intervals between injections remain to be determined.

- 3. In hamster no. 4 it required relatively much more drug to eliminate the residue of the infection than the bulk; 8 injections (80 mgm.) removed by far the greater part of the infection, and a further 7 injections did not sterilize. This was also marked in hamster no. 11 treated with urea stibamine; 10 injections (200 mgm.) destroyed the overwhelming majority of the parasites, and a further 8 injections (160 mgm.) did not sterilize the remaining slight infection.
- 4. There does not appear to be an appreciable amount of destruction of parasites in the first 6 days of treatment, even when big doses are used.

ACTION OF FLAGELLATES IN VITRO

The strain used for producing the infection in the hamsters was grown on 10 per cent. rabbit serum in Locke solution with 0.1 per cent. glucose and varying concentrations of 4:4'-diamidino stilbene. It was found that 1:20,000 4:4'-diamidino stilbene completely inhibited growth, while in 1:30,000 there was a slight growth. L. donovani is not inhibited by 1:10,000 tartar emetic, and there is survival of flagellates even in 1:5,000. The above medium made up with 1:200 neostibosan is quite suitable for growth of L. donovani. We cannot, of course, make deductions from in vitro experiment on the flagellate stage in cultures on the mode of action of the above drugs under the very different conditions under which the L.D. body lives and multiplies in vivo.

CULTURES ISOLATED FROM TREATED ANIMALS

The cultures isolated from treated animals at a time when the infection was vanishing were very different in composition from the normal parent culture used for infecting the hamsters. They consisted mainly of the type of flagellate designated as 'short forms', i.e., thin flagellates, body $4-10\mu$, with a flagellum longer than the body. Larger forms with a body $15-20\mu$ or more were absent. After two subcultures these characters did not alter, but after three subcultures there was an approach in composition of types to those of the parent culture.

SUMMARY AND CONCLUSIONS

4:4'-diamidino stilbene in repeated doses of from 2.5 to 40 mgm. per kilo. body weight has a marked therapeutic action on infections of *L. donovani* in the Syrian hamster.

In three animals sufficiently studied the infection was sterilized by 27 injections of 2.5 mgm. per kilo. body weight, 24 injections of 10 mgm. per kilo. body weight, and 10 injections of 20 mgm. per kilo. body weight.

This is the first instance of a drug not containing antimony with a chemotherapeutic action on infections of *Leishmania donovani*.

The histological changes in the spleen during the course of treatment are described.

REFERENCE

LOURIE, E. M., and YORKE, W. (1939). Studies in chemotherapy. XXI: The trypanocidal action of certain aromatic diamidines. Ann. Trop. Med. & Parasitol., 33, 289.

EXPLANATION OF PLATE IV

- Fig. 1. Hamster no. 4. Spleen at commencement of treatment. Note megakaryocytes and large number of parasites. $(\times 520.)$
- Fig. 2. Hamster no. 4. Spleen after 15 injections each of 10 mgm. per kilo. body weight. No parasites found in smears or sections, but cultures positive. Megakaryocytes disappeared. (× 520.)
- Fig. 3. Hamster no. 4. Same as fig. 1, \times 1,500.



Fig. 1



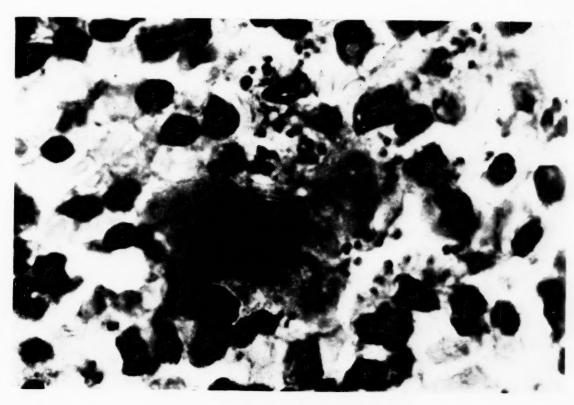


Fig. 3



EXPLANATION OF PLATE V

- Fig. 1. Hamster no. 6. Spleen before treatment. (× 135.)
- Fig. 2. Hamster no. 6. Same spleen 33 days after commencement of treatment. Cultures negative. (Eight days after previous laparotomy.) Note marked decrease in cells in red pulp. (× 135.)

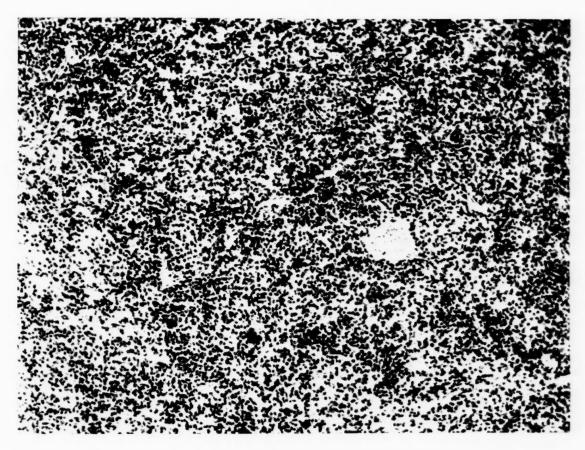


Fig. 1

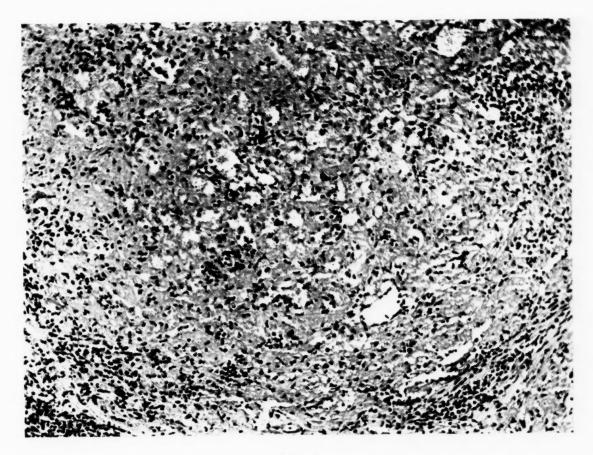
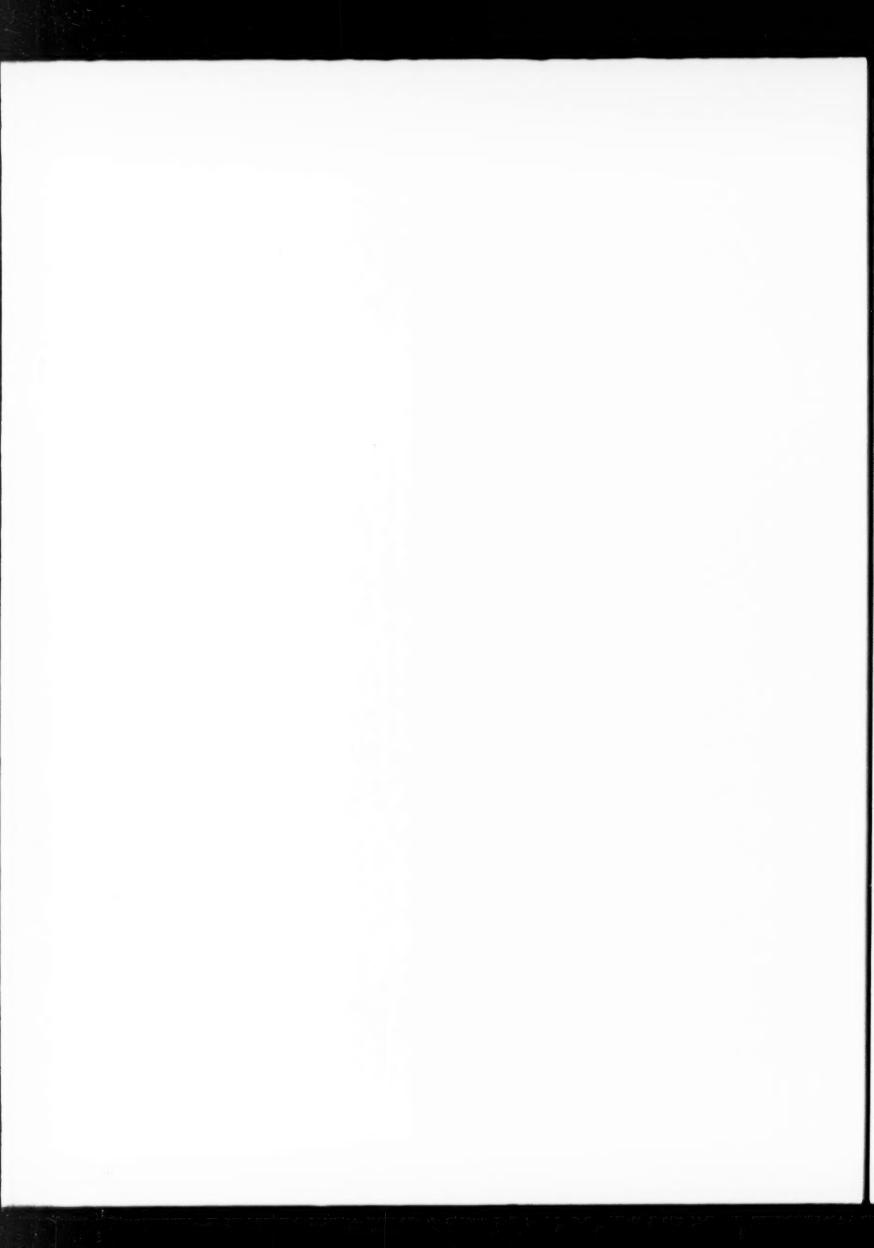


Fig. 2

H. R. Grubb, Ltd., Poplar Walk, Croydon





STUDIES IN CHEMOTHERAPY

XXIII.—A CASE OF INDIAN KALA-AZAR TREATED WITH 4:4'-DIAMIDINO STILBENE

BY

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(Received for publication September 24th, 1939)

This note records the result of treating a case of Indian kala-azar with an aromatic diamidine, viz., 4:4'-diamidino stilbene—R-CH: CH-R (Lourie and Yorke, 1939) We were induced to test this compound on a human case of leishmaniasis by private communications we had received from Dr. Adler, to whom we had sent a quantity of the substance with the object of ascertaining whether it had any action on Leishmania infections in hamsters (see Adler, 1939).

The patient, a Hindoo seaman aged 31, came from Calcutta, and was first seen by us at a local provincial hospital where he had been an in-patient for the previous three weeks, suffering from irregular fever of unknown cause. In view of his inability to speak English, it was impossible to obtain a history, other than that he had come on a voyage from India, and had had 'fever' for two or three months. He was transferred to the Tropical Ward of the Liverpool School of Tropical Medicine for investigation and treatment.

On admission on June 24th, 1939, the temperature was of a remittent type, ranging daily two or three degrees to 103° F.; the patient appeared exceedingly ill, he was much emaciated, and weighed 42·7 kilo. The spleen was firm and tender, reaching the umbilicus; the liver was enlarged, extending two fingers below the costal margin, and was tender on pressure.

The blood picture was as follows:

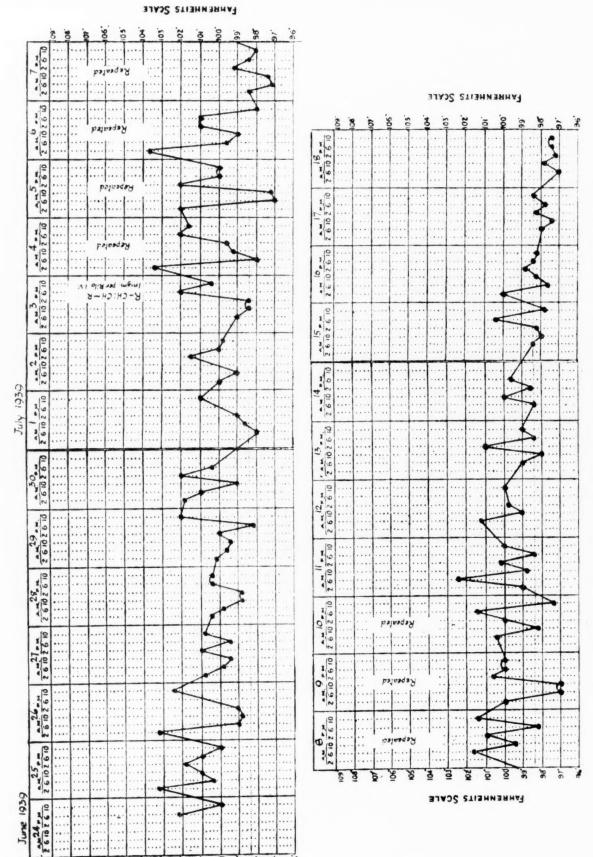
Red cells . . 3,500,000

Haemoglobin 70 per cent.

Leucocytes . . 2,500

Blood sown on Locke-blood-agar medium gave a culture of *Leishmania* donovani, and Leishman-Donovan bodies were found in fair numbers in sternal marrow smears.

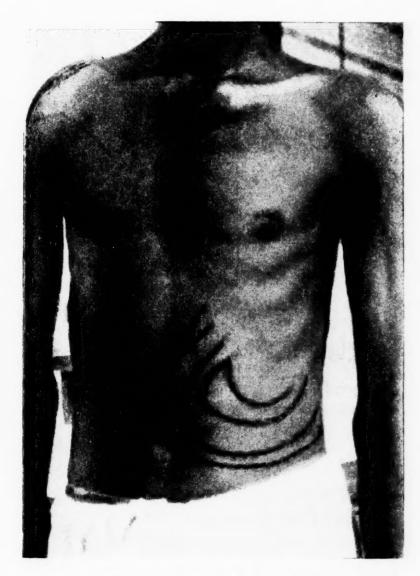
On July 3rd, 1939, a course of injections with 4: 4'-diamidino stilbene was begun. The dosage was 1.0 mgm. per kilo. daily intravenously for 8 days, and the total amount of drug injected was 360 mgm.



FAHRENHEITS SCALE

Temperature-chart of case of Indian kala-azar, indicating period of treatment.

The first few injections appeared to aggravate the symptoms, the febrile excursions becoming greater and the general condition deteriorating. Soon, however, the patient's general health appeared to improve, but the weight continued to decline and there was no appreciable effect on the temperature or



Silver nitrate markings on the skin, showing the lower borders of the liver before treatment; and of the spleen before, during and after treatment with 4: 4'-diamidino stilbene.

Lowest lines: July 1st and 14th, 1939 (treatment began on July 3rd, and was completed on July 10th, 1939).

Other lines: July 20th, 1939.

27th,

on the size of the spleen or liver during treatment. It was not until two days after the completion of treatment that the temperature began definitely to decline. It finally became normal six days after the end of treatment and remained normal during the two and a half months the patient continued to be

under observation. With the fall in temperature, the general condition improved markedly and the weight, which by July 22nd had fallen to 37.5 kilo., began to increase.

Ten days after the last injection it was noticed for the first time that the spleen had commenced to shrink. From this point onwards the shrinkage was so astonishingly rapid that daily differences could actually be observed. By the 17th day after treatment the size of the spleen had so decreased that the organ was just palpable below the costal margin. The size of the liver decreased coincidently with that of the spleen.

On July 20th, 1939, that is, 10 days after the course of treatment, the blood count was as follows:

Red cells .. 4,200,000 Haemoglobin 75 per cent.

Leucocytes . . 5,400 Neutrophils 70 per cent. Eosinophils . . 3 ,, ,,

Basophils . 1 ,, ,, Lymphocytes 15 ,, ,,

Mononuclears 11 ,, ,,

Culture of the blood on Adler's medium (Locke-blood-agar) was negative and inoculation into hamsters did not produce infection. No parasites could be found in the sternal marrow; cultures in Adler's medium were negative, and hamsters inoculated with the marrow did not become infected.

The patient's general condition steadily and progressively improved, and when he was discharged from hospital on September 23rd (two and a half months after the end of treatment) his weight had increased to 46.5 kilo. and he appeared to be perfectly well.

SUMMARY

This note records the apparent cure of a case of Indian kala-azar by an aromatic diamidine, viz., 4:4'-diamidino stilbene.

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A NOTE ON THE TREATMENT OF A CASE OF LEISHMANIA INFANTUM WITH 4:4'-DIAMIDINO STILBENE

BY

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(Received for publication November 9th, 1939)

After the curative effect of 4:4'-diamidino stilbene (see Lourie and Yorke, 1939) on infections of *Leishmania donovani* in Syrian hamsters had been demonstrated (Adler and Tchernomoretz, 1939), we had an opportunity of testing the drug on a human infection with *L. infantum*.

CASE HISTORY

H.S., female, aet. 32, born in Palestine and had never left the country. Was quite well till the summer of 1938, when she had an attack of dysentery (type not known). In March, 1939, complained of fever, vomiting and dizziness, and was confined to bed for 17 days. On March 30th, 1939, was admitted to the Government Hospital, Haifa. Spleen puncture revealed numerous L.D. bodies, and she was given the following course of stiburamine:

April 9th, 1939 0.05 gm. April 11th, 1939 0.15 ,, April 13th, 1939 0.2 ,, April 15th, 1939 0.2 ,, April 17th, 1939 0.1 ,, April 20th, 1939 0.1 ,,

On April 26th she was discharged from hospital free from temperature and in good condition. Three weeks after discharge she again complained of fever. She was readmitted to the Government Hospital, Haifa, on June 1st. On June 5th spleen puncture again revealed L.D. bodies. On June 28th the patient, who has received no further treatment, was transferred to the Rothschild Hadassah University Hospital.

CONDITION ON ADMISSION

The patient complained of debility, fever, blurred vision, tinnitus and loss of appetite. Weight 59 kilo. The skin and mucous membranes were yellowish-white. The spleen was very hard and tender, and reached two fingers below the umbilicus. Border of liver slightly below costal margin.

Dr. A. Feigenbaum, Ophthalmic Surgeon to the Rothschild Hadassah University Hospital, kindly examined the patient and reported nothing abnormal in the retina.

The blood picture on admission was as follows:

Red cells . . . 2,200,000
Haemoglobin 55 per cent. Sahli
Leucocytes . . 2,600

Neutrophils . . 59 per cent.
Eosinophils . . 3 ,, ,,
Lymphocytes . . 33 ,, ,,

Large mononuclears 5 , , ,

Sedimentation-rate: 20 minutes (Linzenmeier).

Urine: no pathological findings.

Sternal puncture: no L.D. bodies found in smears, but cultures on Locke-blood-agar were positive (5 tubes out of 5).

Temperature: irregular; the peak varied from 37.4 to 39.6° C.

TREATMENT

July 5th, 1939: 60 mgm. 4:4'-diamidino stilbene in 120 c.cm. saline intravenously.

July 7th, 1939: 60 ,, ,, ,, 100 ,, ,, intravenously.

July 9th, 1939: 100 ,, ,, ,, ,, 30 ,, distilled water intramuscularly. The injection was rather painful.

July 11th, 1939: Ditto. The patient had diarrhoea.

July 13th, 1939: Ditto. The patient complained of diarrhoea and abdominal pain. The stool contained blood, mucus and numerous *Entamoeba histolytica*. The treatment with 4:4'-diamidino stilbene was stopped until July 21st.

From July 13th to July 18th inclusive the patient received 8 injections each of 0.05 gm. emetine. The diarrhoea and abdominal pain subsided and *E. histolytica* was not found in the faeces, but the temperature was not affected by the emetine treatment.

Between July 21st and September 7th a further 19 intravenous injections each of 100 mgm. in 30 c.cm. distilled water were administered at intervals of two, or occasionally three, days. The injections were not followed by any

untoward effects, and produced no noticeable change in blood-pressure or pulse-rate.

On August 8th (after 12 injections) the temperature became normal and has since remained normal, but the treatment was continued because the spleen was not noticeably diminished in size. Two weeks later it had shrunk to the level of the umbilicus.

On September 7th bone-marrow from the sternum was sown on Lockeblood-agar, and cultures were negative during an observation-period of one month.

Blood changes during treatment:

July 16th, 1939 Red cells: 2,900,000 Leucocytes: 3,400 July 23rd, 1939 Red cells: 2,900,000 Leucocytes: -3,600July 30th, 1939 Red cells: 3,500,000 Leucocytes: 2,800 Aug. 10th, 1939 Red cells: 3,900,000 Leucocytes: 4,800 Sedimentation-rate: 43 minutes Aug. 15th, 1939 Aug. 21st, 1939 Red cells: 4,500,000 Leucocytes: 6,200 Aug. 29th, 1939 Red cells: 3,900,000 Leucocytes: 6,000 Sept. 5th, 1939 Sedimentation-rate: 50 minutes Sept. 12th, 1939 Red cells: 4,300,000 Leucocytes: 5,600 Sedimentation-rate: 2 hours, 20 minutes

On September 13th, the patient was discharged from hospital and observed as an out-patient.

> Sept. 25th, 1939 Red cells: 4,400,000 9,200 Leucocytes:

Three fingers above umbilicus. Spleen: Sept. 6th, 1939 Sept. 13th, 1939 Four fingers above umbilicus.

> Oct. 8th, 1939 Five fingers above umbilicus (three fingers below costal margin).

Formol-gel positive (gelification after one hour).

GENERAL CONDITION

By October 8th the patient had gained 3.5 kilo, and appeared in excellent condition. Her complexion had changed from a pale yellow to a healthy red. Tinnitus and blurring of vision, probably caused initially by the anaemia, had disappeared.

REMARKS

Relapse cases of Mediterranean visceral leishmaniasis are notoriously difficult to treat, particularly if the spleen, as in this case, is both large and hard. In this type of case the severity of the clinical condition often shows no relation to the number of parasites, which may be relatively few.

In our case the temperature did not become normal till five weeks after the commencement of treatment. There was a marked improvement in the red count three to four weeks, and in the leucocyte count six weeks, after the commencement of treatment, but the sedimentation-rate, always rapid in kala-azar, approached normal much later (ten weeks).

Repeated intravenous injections of 4:4'-diamidino stilbene 1.7 mgm. per kilo. at intervals of two days produced no ill effects of any kind. It is interesting to note that amoebic dysentery appeared during the course of treatment.

In view of the clinical severity of the case, the size and hardness of the spleen, and, above all, the fact that we are dealing with a relapse case, the result must be regarded as satisfactory.

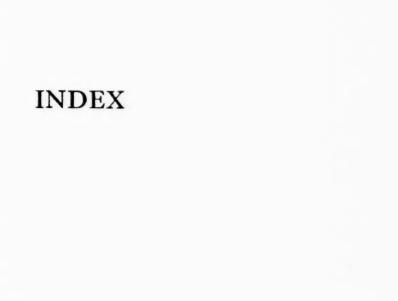
The above findings, together with the proved curative effect of 4:4'-diamidino stilbene on *Leishmania donovani* in hamsters, indicate that this drug is a valuable aid in the therapy of visceral leishmaniasis.

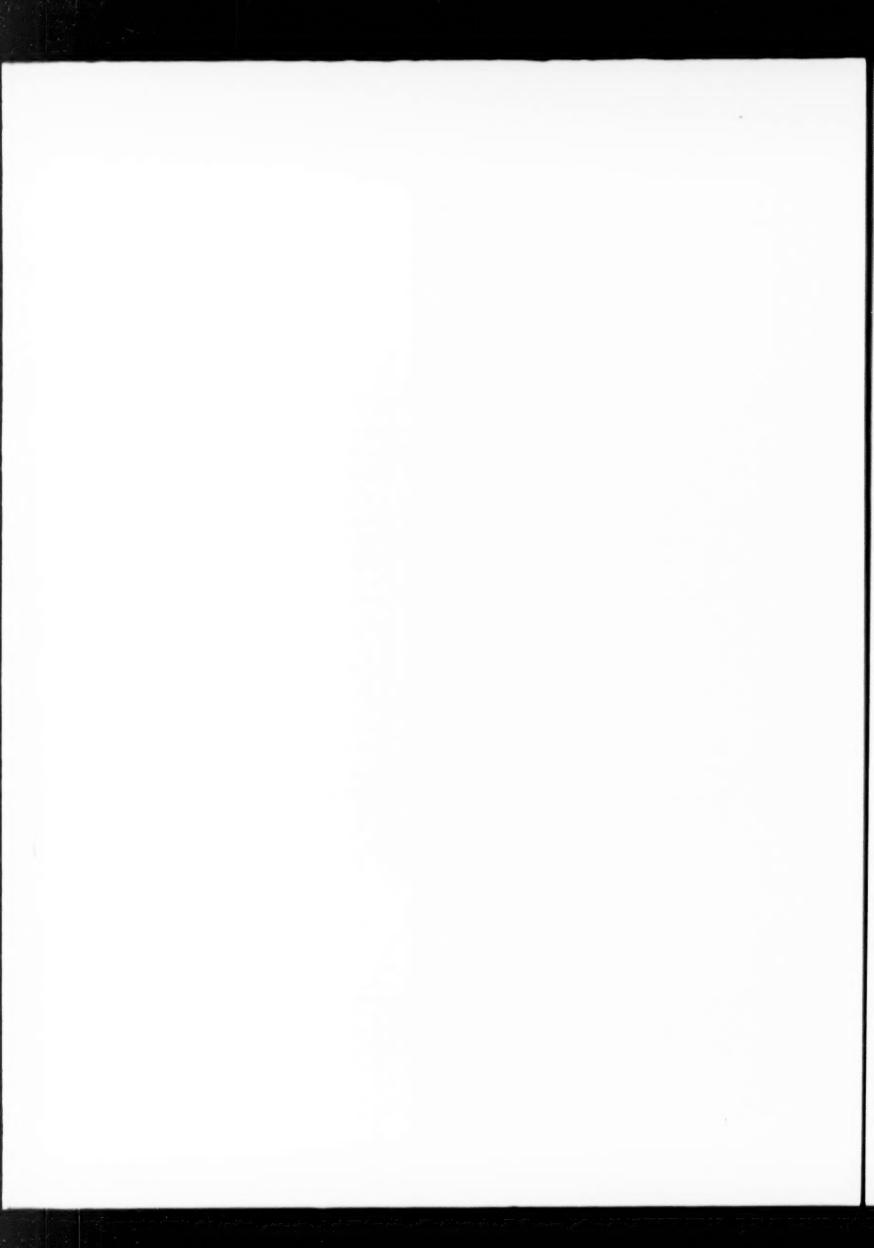
Acknowledgements.—We have to thank Dr. J. Shapiro, of the Government Medical Service of Palestine, for bringing this case to our notice; Professor Warrington Yorke, F.R.S., of the Liverpool School of Tropical Medicine, for kindly supplying us with the drug for therapeutic trials; and Dr. H. Yaski, Director of the Rothschild Hadassah University Hospital, for his interest and kind permission to test the drug on a human case.

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